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Olive fly management today: the role of predators

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Dedication

My dissertation is dedicated to my grandmother Lena (contraction of Madalena), who died in the beginning of this year and didn't got to see me finish my masters. She always wanted more for herself and her grandchildren. She had a very difficult life and still found joy in the small things and in her routine. I hope she is finally at peace.

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Resumo

O olival é uma cultura de grande relevância socioeconómica para a região mediterrânica. A procura e venda de azeite tem aumentado ao longo dos anos, pelo que a sua produção também cresceu, aumentando os tipos de cultivo intensivos e superintensivos. Estas práticas aumentam a utilização de pesticidas para o controlo das pragas associadas à olivicultura, sendo a sua praga primária a mosca da oliveira (*Bactrocera oleae*). Paralelamente, tem se observado um aumento de alelos de resistência a estes químicos nas populações de mosca de azeitona e muitos destes produtos estão a ser banidos ou têm elevadas restrições de uso a nível europeu. Do lado dos consumidores, observa-se atualmente uma preferência por produtos de origem biológica. Face a estas constrições na produção tem-se procurado alternativas para o controlo da sua principal praga, entre eles, o uso de controlo biológico. Este tipo de gestão utiliza vários organismos auxiliares, sendo o foco deste trabalho os artrópodes predadores, por ser uma linha de investigação em ascensão e existirem ainda poucos estudos.

O objetivo principal deste trabalho foi a identificação de possíveis predadores artrópodes da mosca da oliveira na região do Alentejo durante o Outono de 2016, devido às características biológicas de *B. oleae* durante esta estação, em que simultaneamente ocorrem adultos e pupas. A amostragem foi realizada tanto na copa das oliveiras como nas plantas espontâneas no solo. Além da identificação morfológica e molecular de potenciais predadores, também se caracterizaram as amostras em termos de diversidade ecológica, riqueza e abundância. Procedeu-se ainda à análise do conteúdo do sistema digestivo das espécies mais promissoras de predadores numa tentativa de identificação de mtDNA da mosca da oliveira, confirmando assim o seu consumo em ambiente natural.

Foram identificadas 177 espécies predadoras ou generalistas, das ordens: Araneae, Coleoptera, Heteroptera, Hymenoptera, Mantodea, Neuroptera, Opiliones e Pseudoscorpiones. As ordens com maior frequência, abundância e diversidade foram Araneae e Hymenoptera tendo sido as escolhidas para efetuar a identificação molecular e a analise do trato digestivo. Na identificação molecular foram usadas 10 formigas e 29 aranhas. Devido a contaminações e a limitações de tempo não foi possível confirmar por análise molecular a identificação de todos os exemplares escolhidos. Em relação à análise do conteúdo do sistema digestivo das espécies de formigas estudadas (*Tapinoma* sp.1 (*nigerrimun-simrothi* complex), *Plagiolepis pygmaea* e *Crematogaster scutellaris*), houve resultados positivos para *Bactrocera oleae*, confirmando que estes predadores consomem a potencial praga. Já nas aranhas testadas, não foi encontrado DNA da mosca da oliveira, mas pode tratar-se de um falso negativo, dado que os métodos que se usam atualmente podem não a ter detetado ou podem ter sido capturados quando tinham já digerido completamente a presa.

Em termos da caracterização ecológica, a maioria das amostras revelaram valores baixos nos índices de diversidade, abundância e riqueza específica, com algumas exceções. Sendo o objectivo do trabalho uma imagem da diversidade de predadores existentes, a amostragem não foi desenhada para possibilitar relacionar robustamente fatores climáticos e bióticos (como o tipo de espécies de plantas espontâneas). Quanto à caracterização das espécies nas amostras de oliveira, a morfoespécie mais frequente foi *Philodromus* sp.1 com o valor de 38.9%, a mais abundante *Crematogaster scutellaris* com um valor de 129.0 e a dominante foi *Chrysoperla carnea* com 82.9%. No caso das amostras recolhidas em plantas espontâneas, a espécie mais frequente e mais abundante foi *Plagiolepis pygmaea*, com uma frequência de 55.8% e uma abundância de 230.0. Neste caso houve 3 espécies/morfoespécies dominantes, todas elas com 100.0% de dominância – *Cunctochrysa* sp., *Ameles spallanzania* e Opiliones Morfotipo sp.1. Há que referir que estas morfoespécies foram as dominantes por serem as únicas representativas das suas ordens nestas amostras.

Tendo em conta os resultados obtidos, foram sugeridos métodos com base em bibliografia para o aumento e promoção destes artrópodes no olival, nomeadamente o tipo de gestão e o de cultivo

(biológico, intensivo e superintensivo) praticados na cultura influenciam a abundância e diversidade de artrópodes. Também é referido na bibliografia, que o uso excessivo de pesticidas diminui a abundância das espécies, mesmo os homologados e permitidos nas produções biológicas. A presença de plantas espontâneas perto das culturas tem efeitos benéficos na presença de artrópodes, assim como o tipo de plantas presentes. Para a promoção de aranhas é importante a presença no ecossistema de alimentos não presa, como pólen ou mel, pois são importantes para a sobrevivência das aranhas imaturas, assim como a existência de rochas no solo das culturas tem efeitos benéficos neste grupo, pois facultam-lhes locais para hibernarem e esconderem-se, permitindo um aumento da sua abundância e diversidade.
Sugere se que se prossiga a identificação de predadores da mosca de oliveira no Outono, mas redesenhando a amostragem de modo a incluir registos de variáveis ambientais, aumentando a frequência da monitorização nas oliveiras e nas plantas espontâneas. Recomenda se também que a identificação morfológica seja complementada por análises moleculares, e se amplie as análises do trato digestivo a outras espécies, para incrementar o conhecimento dos predadores de *Bactrocera oleae*.

Palavras-chave: *Bactrocera oleae*; controlo biológico; olival; predadores.
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Abstract

The olive crop has great socioeconomic importance in the Mediterranean basin. With the increase in demand, its farming was also intensified, as well as the chemical control of its main pest – *Bactrocera oleae*. This control is so far mainly based on dimethoate, a chemical which use in Europe is being highly restricted. Moreover, a rise in dimethoate resistance in the olive fly populations of the Mediterranean basin has been observed. Altogether, and associated with consumers mindset change, there is a bigger demand for biological products. Consequently, other methods of management of this pest, as biological control, are expanding. The main objective of this work was the morphological identification of natural enemies of the olive fruit fly during the Autumn 2016 in samples of olive canopy and ground cover. Additionally, the sample's ecological characterization and molecular identification of selected morphospecies was performed. As a proof-of-principle of predation, the gut content of a selected number of predators was probed molecularly, using specific primers, to confirm the predation on *Bactrocera oleae*. Several morphospecies with predatory interest, from the orders Araneae, Coleoptera, Heteroptera, Hymenoptera, Mantodea, Neuroptera, Opiliones and Pseudoscorpiones, were identified. The morphospecies of the Araneae and Hymenoptera order had the greatest diversity, abundance and frequency. Of the 39 putative morphospecies selected for molecular taxonomy, due to lack of amplification/specificity and/or time constraints, only 12 were sequenced at COI amplicon. Two morphospecies were not correctly identified, and some remain not confirmed due to lack of information on the databases. For the gut analyses, a positive result was found in the tested Hymenoptera, showing the effective predation of ants on olive fruit fly, but not in the gut of the Araneae. This result does not exclude the possibility of Araneae preying in *B. oleae*, since it is possible the methods used might not detect the DNA or the consumed prey degraded faster than the frame time to detect it. The samples were characterized in terms of ecological indexes, abundance and richness. In both set of samples – olive canopy and ground cover – the values of diversity, abundance and richness were very low, with a few exceptions. The morphospecies with the highest frequency in olive canopy was the spider *Philodromus* sp.1, the more abundant was the ant *Crematogaster scutellaris* and the dominant was the common green lacewing, *Chrysoperla carnea*. At the ground cover samples, the more frequent and abundant morphospecies was the ant *Plagiolepis pygmaea* and the dominants were the European dwarf mantis *Ameles spallanzania*, the green lacewing *Cunctochrysa* sp. and the Opiliones Morphotype sp.1. In this study some suggestions to increase the diversity and abundance of natural enemies and to promote the management of the olive fruit fly are discussed.

Key-words: *Bactrocera oleae*; biological control; olive crop; predators.

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1. Introduction

1.1. The importance of the olive tree

The olive is important to the Mediterranean basin since ancient times. Evidence of olive cultivation in Greece dates back 3.500 years, after spreading through all the entire Greek speaking world and later, with the rise of the Roman empire, to the entire Mediterranean basin (Loumou, 2003).

According to Eurostat (Eurostat, 2019), the Mediterranean basin has many varieties of olive trees and this region alone produces 99% of the world olive oil and consumes around 87% of it. Thanks to its high value per unit, accounts for 19% of the value of world trade in edible vegetable oils. In 2018 the EU exported over 1.6 million tonnes of olive oil worth 5.7 billion euros (63% went to other EU members). This represents an increase of 15% since 2013. And the EU members imported 1.2million tonnes of olives worth 3.9 billion euros (85% of these imports went to other UE members). There was an increase of 10% compared with 2013. Overall, the economy around olive oil has been increasing. The major producers of olive oil and olives are UE members are Spain, Italy, Portugal and Greece.

As Portugal is one of the major producers in the world (third position within EU, with 56000 tonnes a year which correspond with 10% of all UE production), this olive grove has a high relevance to the economy of the country, mainly in the Alentejo region. Considering the information from the Portuguese Institute of Statistics (INE, 2018), Alentejo region is the biggest producer of olives in Portugal, with 551380 tonnes of olives for olive oil being produced in 2018, against the 109404 tonnes produced in the Norte region. Also, in terms of land use in Alentejo region, the olive is the one that has more land dedicated to it, 184936 ha against the second biggest crop in the region, grapes for wine production which uses 32368 ha of land (INE, 2018).

With the growing importance of this crop in the EU and around the world, and to face the public demands of more olive products, the olive crops have been changing and nowadays, three main production schemes are recognized, according to the cultural and management system: traditional, intensive and super intensive. Traditional agricultural production is defined the use of simple technology allied with methods transmitted from generation to generation by word of mouth or practice. Generally practiced by small communities and operations tend to follow one another in sequence with the output of work strongly influenced by annual and ceremonial cycles and has as objective the production of enough product for the subsistence of the community (Cochrane, 1975). In the traditional olive grove, trees are spaced with densities from 60 to 200 trees per hectare and are mainly rainfed (without irrigation). Intensive agricultural production is characterized by the use of irrigation and synthetic fertilizers and pesticides, cultivation of a few high yielding varieties, high and continued mechanization with the reduction or removal of seminatural habitats in farm areas. On average, trees have a density from 280 to 450 trees per hectare and the objective is high production of the crops to export and sell the products (Behera, 2016). The super intensive system has an even higher tree density, around 1 500 to 2 200 trees per hectare, and production is high and fast (Olint, 2018).

The olive crop can also be classified according to the type of management regarding pest and diseases: conventional, organic and integrated management. The conventional management has many traits similar to intensive farming and is put into practice both in traditional and intensive or super intensive olive groves. It is characterized by rapid technological innovation, large scale farms with monoculture crops, high mechanization of farm work, extensive use of pesticides and fertilizers and dependency in agribusiness (Fisher, 2017). Organic management uses the innovation of technology and most recent methods to have similar production levels of the conventional management but only using fertilizers and pesticides of natural occurrence and some techniques used in traditional agricultural production such has crop rotation, companion planting, among others (Gold, 2007; Martin, 2009). It attempts to look at the ecosystem as an all focusing on improving the soil microbiology and ecosystem services as a way

of promoting plant growth and produce yield (Watson, 2002). According to the International Organisation for Biological and Integrated Control-West Palaearctic Regional Section (IOBC-WPRS, 2004), integrated pest management is a farming system with the objective of producing high quality food and other products by using natural resources and regulating mechanisms to substitute damaging inputs and secure sustainable farming. It has as objective to improve and preserve the soil fertility, environmental diversity and the promotion of ethical and social criteria. The diversity of methods used in farming (technical, biological and chemical) are carefully managed taking into account the profitability, environmental protection and social requirements (IOBC/WPRS, 2004).

1.2. Olive fruit fly, the main pest of olive groves

All of these systems of agricultural production and methods of management are used in olive crops. Even though they vary greatly in the way they deal with their production, some of those challenges are transversal to the management type namely disease and pest management. Pest management is crucial to the quality and quantity of production. In the case of olive tree and its fruit there are some important pests that if they are not in check can cause huge losses in production.

The pests that are related to the olive tree and production are: olive psyllid (*Euphyllura olivina* (Costa, 1839)), black scale (*Saissetia oleae* (Olivier, 1791)), olive bark beetle (*Phloeotribus scarabaeoides* (Bernard, 1788)), tabby knot-horn (*Euzophera pinguis* (Haworth, 1811)), olive fruit fly (*Bactrocera oleae* (Rossi, 1790)), olive moth (*Prays oleae* (Bernard, 1788)), jasmine moth (*Margaronia unionalis* (Rossi, 1794)) and olive thrip (*Liothrips oleae* (Costa, 1857)). Worldwide the most relevant ones are the olive fruit fly, the olive moth and the black scale, being the first species the main responsible for production damage in the Mediterranean region. In Alentejo region, the most concern goes to the activity of the olive fruit fly, responsible for losses up to 80% in olive oil production and 100% of some table cultivars (Daane, 2010), besides having indirect effects in the quality, composition and properties of the olive oil, causing other type of production losses (Nobre, 2019), even though the other two species may occasional be of relevance. Due to its relevance, this work will focus on the olive fruit fly.

1.2.1. Morphology

The olive fruit fly is part of the subfamily Dacinae and tribe Dacini, which contains primarily Afrotropical, Australasian and Oriental species. *Bactrocera oleae* has genetically distinct sub-Saharan African, Mediterranean and Pakistani populations (van Asch, 2012). The species is of African origin, where its original hosts are precursors of the cultivated olive tree (Nardi, 2005). The invasion of this fruit fly to the cultivated olive trees in Africa, was passed on to the Mediterranean orchards and the more recent invasion in the Americas has its origin from the Mediterranean ones (Daane, 2010).

Eggs size is about 0.74 mm long and 0.21 mm wide with the typical shape of the tephritids fruit flies' eggs – elongated and a bit curved on the middle. When they are freshly deposited, they present an opaque white creamy colour (Genc, 2014).

B. oleae larvae are small - around 5-6 mm long and 1.5 mm wide, elongated and slightly tapered at each end (Philips, 1946). The larva has a conical narrow front and develops in 3 instars. The 3 instars can be distinguished by their cephalopharyngeal structures. The first instar is metapneustic, equipped with one pair of posterior stigmas. The second and third instar can be distinguished by the different shapes that the frontal stigmas can assume. The pupa is 3.5-4.5 mm long, varying from creamy white to yellow-brown colour. This change in colour can help determine the pupas age (Raspi, 1998).

The olive fruit fly adult is 4 -5 mm long. They present a small dark spot in the apex of the wings and a narrow, elongated anal cell. The compound eyes are big with violet-green or blueish-green colour. The mesonotum is bluish-grey with 3 black longitudinal lines (Raspi, 1998). This pattern may vary depending on the location of the population (Bon, 2015). The abdomen is light brown with variable colourings. Usually there are pairs of black bands in the laterals of the first to forth tergit (Raspi, 1998).

The species presents sexual dimorphism, being the females larger than the males with a prominent ovipositor (Mendes, 2017).

1.2.2. Life cycle

In the broader spectrum, dacine fruit flies are diurnal insects, resting in the undersides of leaves of their host plants in the night time. Their activities during the daytime can be divided into 5 functional categories: feeding, mating, ovipositing, dispersing and resting. The time dispended in which activity depend on varied factors has age, sex, availability of mates, availability of hosts, short-term climatic conditions and long-term ones (Fletcher, 1987).

The general consensus regarding the feeding type done by *B. oleae* is monophagous frugivore, since it only feeds on the fruit of a few *Olea* species. The fruit olive fly eggs are laid inside the olive fruits and after inclusion, the neonate larva feeds on it (Tzanakakis, 2003). While the larva of olive fruit flies is completely dependent on the presence of Olea species fruits, the adult was observed feeding on other organic sources just as insect honeydew, plant nectar, plant pollens and fruit exudates. They may also feed on bird dung, bacteria and yeasts to meet their nutritional requirements (Tsiropoulos, 1977; Tsiropoulos, 1984). The existence of these other sources of food for the adult insect is critical for their survival and reproduction during the periods of the year when olives fruits are not available.

The female usually deposits 1 egg per olive fruit (Ant, 2012) and tend to do it in smaller fruits, with less than 1 cm^3 (Yokoyama, 2006). The eggs take 2 to 3 days to hatch (Nardi, 2003). Both the egg and larval development are temperature dependent (Genc, 2008; Tsitsipis, 1977).

The larva, after hatching, stays inside the olive fruit and moves to the deeper part to start feeding. The larval stage lasts around 20 days (Rice, 2000). Pupation of this species can occur in the olive fruit or the soil and that is dependent of the time of the year and the number of generations, being pupation in the soil, more common during the winter months or when there are more generations per year (Rice, 2000). Pupation in the soil, mainly when it occurs in the winter can take around 6 months and when it happens on the olive fruit can last between 8 to 10 days (Vossen, 2006). The adult lives around 6 months and a female during her lifetime can lay as many as 500 eggs (Rice, 2000).

Overall, olive fruit fly development and the resulting number of annual generations is dependent of ambient temperature (already referred above), humidity, microclimate within the olive canopy and on the availability and quality of the olive fruit (Burrack, 2008).

1.2.3. Impact on olive orchards

There are several ways in which the olive fruit fly can reduce and impact negatively the olive production. One of them is when immature fruit are stung by female fruit flies, they may be aborted prior to harvest (Tzanakakis, 2006).

They can impact the varieties destined to the table, since larval consumption of fruit pulp has been estimated to range from 50 to 150 mg per larva, depending on the variety. Since no consumer wants to find marks or larva in the fruit, the economic threshold level for producer is near 0 larva per fruit, so therefore olive table varieties receive more attention to the infestation of *B. oleae* (Daane, 2010). In comparison, olive fruits destined for oil pressing are allowed to have higher levels of fruit fly infestation, between 10 to 30% and still be considered acceptable (Neuenschwander, 1978).

There are several factors that may influence the level of impact that *B. oleae* has on the olive oil, including timing and severity of the fruit fly infestation, the variety of olive cultivar, harvest date, the presence of microflora and length of storage time prior to pressing (Pereira, 2004 and Torres-Villa, 2003). The olive fruit time of storage and the level of larval damage, interact synergistically, increasing olive oil acidity. High levels of acidity in olive oil decreases its quality (Gomez-Caravaca, 2008). This relationship between damage and storage time is influenced by the presence of microorganisms such as

bacteria (e.g. *Xanthomonas*), yeasts (e.g. *Torulopsis* and *Candida*) and fungi (e.g. *Fusarium* and *Penicillium*). There is a positive correlation between acidity of the olive oil and the presence of microflora, nonetheless the same can't be said between oils acidity and the level of damage in the olive fruit (Torres-Villa, 2003).

One aspect already referred above in relation to fruit fly infestation is the variety of olive trees. It was found in some studies, mainly California, that female *B. oleae* exhibited strong ovipositional preference for certain varieties of olive cultivars. It was observed that the larva performed better in those preferred ones (Burracks, 2008). On the other side, several studies showed that different varieties of olive cultivars, varied in susceptibility to *B. oleae*. These variations in susceptibility may be due to some factors that play a role in it, like fruit size, weight, colour, fruit epicarp hardness, aliphatic waxes, phenological state of the crop and chemical factors (Iannotta, 2007; Neuenschwander, 1981). These factors are themselves influenced by environment and genetics (Daane, 2010).

One study evaluated several varieties of olive crops and found that the fruit of 2 particular ones – 'Nostrale di Rigali' and 'Nocellara etnea' – when exposed to the fruit fly had low larval infestation and a high percentage of sterile punctures (Iannotta, 1999). Other study by the same author, reported that high amounts of oleuropein and cyanidine present in the fruits of the varieties 'Bardhi Tirana' and 'Tonda nera dolce' respectively, could contribute to low fruit fly infestation (Iannotta, 2007). The susceptibility to *B. oleae* of some local or commonly used varieties of olives is known. For example, 'Verdeal Transmontana', 'Madural' and 'Cobrançosa' are classified as highly susceptible, medium susceptible and less susceptible (Gonçalves, 2012) and it seems that the preference/susceptibility to the fruit fly is related to the volatile compounds produce by the fruits during the maturation process (Malheiro, 2015). Although these studies reveal important information on host resistance to *B. oleae* infestation, they seem to be often neglected and not taken into consideration when designing programs of fruit fly integrated pest management.

1.2.4. Methods of control

With the importance of the olive orchard in the Mediterranean basin and the levels of damage this fruit fly can achieve, the producers need ways of controlling this pest. So far in the last 4 decades, in the intensive and super intensive cultures, the privileged method of control is the use of chemical insecticides, particularly organophosphates (OPs) (Vontas, 2001). The active substance in the more frequent used OPs is dimothoate, which is cheap, soluble in water, which in turn produce few residues in the olive oil and is highly effective in reducing the number of individuals of the fruit fly population in the crop. Nonetheless it's harmful and irritant to humans, animals, auxiliary insects (e. g. *Apis mellifera*) (Volakakis, 2012).

The other problem that has arisen from the over use of OPs, is the increase of the percentage in fruit fly population of resistance genes. Biochemical and molecular analysis of acetylcholinesterase (AChE) – the target enzyme of OPs – and the Ace gene – which encodes it, has led to the identification of mutations in that gene as the underlying cause for OP resistance in *Bactrocera oleae* (Vontas, 2001; Kakani, 2008). Two of these mutations affect amino-acids close to the active site of the enzyme, and are thought to cause alterations in the topology that decrease the effectiveness of the action of OPs (Pereira-Castro, 2015). These mutations were found in very high frequencies in natural fruit fly populations in Greece, Albania and Italy, and slight less frequently in natural populations of France, Spain and Turkey (Nardi, 2006; Baskurt, 2011). A third mutation of a completely different type was later discovered in natural populations of *B. oleae* which reduces the sensitivity to OPs (Kakani, 2008). A more recent study in 2015, found that the two more frequent genetic mutations for OPs resistance were also present in Portugal, reaching more than 50% of the natural populations. Even though the frequency values vary a lot from sample site to site, all of them had the mutations. They could not link if the increase in mutation frequency was due to over use of OPs in the olives orchards or if was imported of the other populations,

like Andalusia (Pereira-Castro, 2015), but in later study in 2019, connected the mutations with the migration hypothesis (Nobre, 2019).
Due to the problems described above and a better knowledge of the ecosystems and care in that regard, scientists, producers and countries, are looking for better management and control of the fruit fly, using an array of other methods, leaving the chemicals ones as last resource or only applied in controlled ways (integrated pest management). Besides chemical control, there is biotechnological and biological control.
There are different types of biotechnological control used in the olive fruit fly including fruit bagging, clipping of infested fruits and the most used one, mass-trapping. This last tactic has the potential to minimize or avoid the use of insecticides and has attracted interest due to their efficacy, specificity and low environmental impact (Navarro-Llopis, 2008).
In mass-trapping, to attract the fruit fly there must be some type of stimulus, which can be chromatic, pheromonal or nutritive. For the pheromonal traps, *B. oleae*'s sexual pheromone – spiroacetal is used (Haniotakis, 1991). Traps for olive fruit fly with food attractant can be used: ammonium bicarbonate salt, dacona, *Dacus* bait, ammonium carbonate salt, modified hexanodiol and ammonium sulphate salt (Broumas, 1994). Some studies are trying other biotechnological methods using SIT (sterile insect technique), MAT (male annihilation technique) and RNA interference (which is a mechanism of gene regulation and an antiviral defence system in cells, resulting in the sequence-specific degradation of mRNAs), but none of them are specific to *Bactrocera oleae*, and use instead other fruit flies (Dias, 2018).
Biological control is a rapid growing area which brings together producers and scientists from many disciplinary backgrounds. The major uses of biological control agents in this case, is biological control of invertebrates' pests using predators, parasitoids and pathogens. The biological control has the aim of reduction in disease or pests through the activity of the biological control agents (Eilenberg, 2001).

1.2.5. Natural enemies

A great relevance to parasitoids has been given in studies about the management of the olive fruit fly. In fact, some countries in the African continent which also produce olive oil have minor economic impact because of the action of native natural parasitoids (Mkize, 2008) like *Psyttalia dacicida* Silvestri, *Psyttalia lounsburyi* (Silvestri), *Utetes africanus* (Szépligeti), *Bracon celer* Szépligeti, *Triaspis daci* (Szépligeti), *Neochrysocharis formosa erythraea* (Silvestri), *Eupelmus afer* Silvestri, *Halticoptera daci* Silvestri and *Coptera silvestrii* (Kieffer) (Daane, 2010). Later authors described more parasitoid species in the region for the olive fruit fly (Neuenschwander, 1982) With the good results obtained in Africa, other studies tried to find or introduce similar species in the regions impacted by *B. oleae*. The braconids typically provide the highest levels of olive fruit fly suppression, including *Diachasmimorpha longicaudata* (Ashmead) and *Psyttalia* spp. [*Psyttalia concolor*, *Psyttalia ponerophaga*, *Psyttalia humilis*, *Psyttalia lounsburyi* (Silvestri)] (Dias, 2018), many of these species are polyphagous parasitoids that may opportunistically attack *B. oleae* (Daane, 2010). Nonetheless the results with parasitoids in Europe have been mixed, with morphological problems (small ovipositors for the larger European olive fruits) by the African parasitoid species to infect the fruit fly in the olive fruit European varieties (Latiere, 1917; Wang, 2009a; Wang, 2009b).
There are some promising results on the use of entomopathogenic fungi as biological control of fruit flies. Particularly in the case of the *Bactrocera oleae*, the use of *Metarhizium anisopliae* Sorokin (Yousef, 2013). The same can be said of entomopathogenic nematodes, where the use of genera like as *Heterorhabditis* spp. and *Steinernema* spp. in the control of larvae and pupae of diverse fruit flies, including the olive fruit fly might have some results (Dias, 2018; Torrini, 2017). The mortality levels varied among the studies, but it's suggested that soil type is a critical factor when selecting the nematode species and planning the fruit fly control strategy (Lezama-Gutiérrez, 2006).

The search for other organisms that can aid in keeping the population numbers under an acceptable threshold remains relevant. Predators, are present all year around and mainly generalists. If being generalists makes them not interesting for classic biological control, their role in supressing population numbers should not be neglected. They are diverse and can cover different trophic guilds. Studies in the Autumn during the bulk phase of pupation follow the assumption that high predation levels on these otherwise defenceless pupae can have a strong impact on the population emerging the following olive season, giving an extra food source for the invertebrates in that time of the year. These studies focused on soil predators, tried to compile species and groups of invertebrates that can and will feed on pupae of *B. oleae*., even though some older ones like Neuenschwander et al. in 1983 already referred some soil predator species: *Carabus* (*Procrustes*) *banonii* Dejean, *Licinus* (*Licinus*) *aegyptiacus* Dejean, *Pterostichus creticus* (I.Frivaldszky von Frivald) (these 3 being carabids), *Ocypus olens* Mueller, *Ocypus fulvipennis* Erichson (these 2 being sthapylinids), *Scolopendra cretica* Attems (Myriapoda), *Aphaenogaster simonelli* Emery, *Crematogaster sordidula* (Nylander) (the more efficient Formicidae predators tested by this study) and *Turdus merula* Linnaeus and *Erithacus rubecula* (Linnaeus) (birds that search the soil for food and could possibly feed on *Bactrocera oleae* larvae) (Neuenschwander, 1983). Dinis et al. (2015) compiled a list of varied invertebrates that may feed on the pupae of the olive fruit fly and confirmed that indeed there is suppression of a part of the pupae population by the soil invertebrates. The invertebrates found were mainly composed by Formicidae, Forficulidae, Araneae, Staphylinidae, Carabidae and Scolopendromorpha. The other studies focused on natural enemies of the olive fruit fly, used carabids and staphylinids: *Calathus granatensis* Vuillefroy, *Pterostichus globosus* (Fabricius) (Dinis, 2016), *Pterostichus melas* (Creutzer) (Panni, 2018) and *Ocypus olens* (O. F. Müller) (Albertini, 2018).

1.3. Objectives

No doubt that olive tree is important to the economy of the Mediterranean basin and with the increased demand in products with olive oil and olives derivates, the management of its key pest – olive fruit fly - is of utmost importance. Since an increase of resistance to the chemicals used for its control is being observed and documented, it is important to access other methods to complement its management, in this case biological control by the existing predators of the ecosystem. Therefore, this dissertation has the objective to:

- Survey and identify the existing predators in the local ecosystem – Alentejo region – separating the predators from soil and olive canopy, since they will in principle predate different stages of the fruit fly (in the olive canopy resides the adult fly, and in the ground the fruit fly is in pupae).
- Characterize the two sets of samples regarding diversity, abundance, presence, richness and dominance to better understand what exists in the ecosystem and eventually stablish possible strategies to increase these species in the region.
- Characterize the sample sites in diversity, abundance and richness with the same objective stated above.
- DNA barcoding of the more dominant species to validate the taxonomical identification.
- Confirm using the more dominant taxa, that the predators identified feed of the olive fruit fly.

2. Methods

2.1. Local Characterization – Alentejo

The Alentejo region, with few exceptions, can be considered a plain due to the erosion of elevations by the flowing waters. The medium altitude is 200 m. The type of climate is Mediterranean, with rain distributed in the region uneven throughout the year; most rain falls in the winter season. The summers are hot and dry. The annual medium precipitation is between 620 mm and 670 mm. The annual medium temperature is 15.8 °C, with considerate monthly variations (MAM, 2013).

It is a region strongly dominated by agricultural and livestock activities. The cultures with more relevance are cereals, orchards, olive, vineyard, cork and pastures for cattle. This region has large forest sectors where the more predominant trees are: cork tree, holm oak, pine tree (MAM, 2013). Alentejo is the main Portuguese region in terms of olive groves, with a national surface area of 52%, and has the higher production of table olives and olives for olive oil (INE, 2017).

2.2. Data collection

Sampling took place in Portugal, in the Alentejo region within the frame of the project ALT20-03-0145-FEDER-000029. A stratified random sampling was designed to cover the region: a grid of 30 x 30 km in a total of 17 squares comprised the stratification of the sampling and inside each square 5 olive areas were selected. All the olives sampled, were organic, as a way to guarantee that pesticides were not applied in recent years (localization of the samples can be observed in Figure 1).

Sampling took place between 25/10/2016 and 15/11/2016, by means of an entomological aspirator (or pooter) powered by a motor (Agricultural Backpack 2-Cycle Aspirator Model 1612, with a 127 mm diameter collection nozzle and 64 km/h air intake, from The John W. Hock Company). At each location, the canopy of 5 random selected trees was sampled for 10 s each, and the collected arthropods pooled into a sampling unit (hereafter referred as local olive sample). When present, ground cover spontaneous plants were also sampled for 50 s, forming another sampling unit (hereafter referred as local weeds sample). Collected samples were preserved in ethanol at 4 ºC until sorting and identification. The main sorting into orders and then into predators, parasitoids and other groups was also performed in the frame of the above mentioned project.

In the sorting process, the insects were transferred to 1.5 ml Eppendorf's with 70% alcohol.

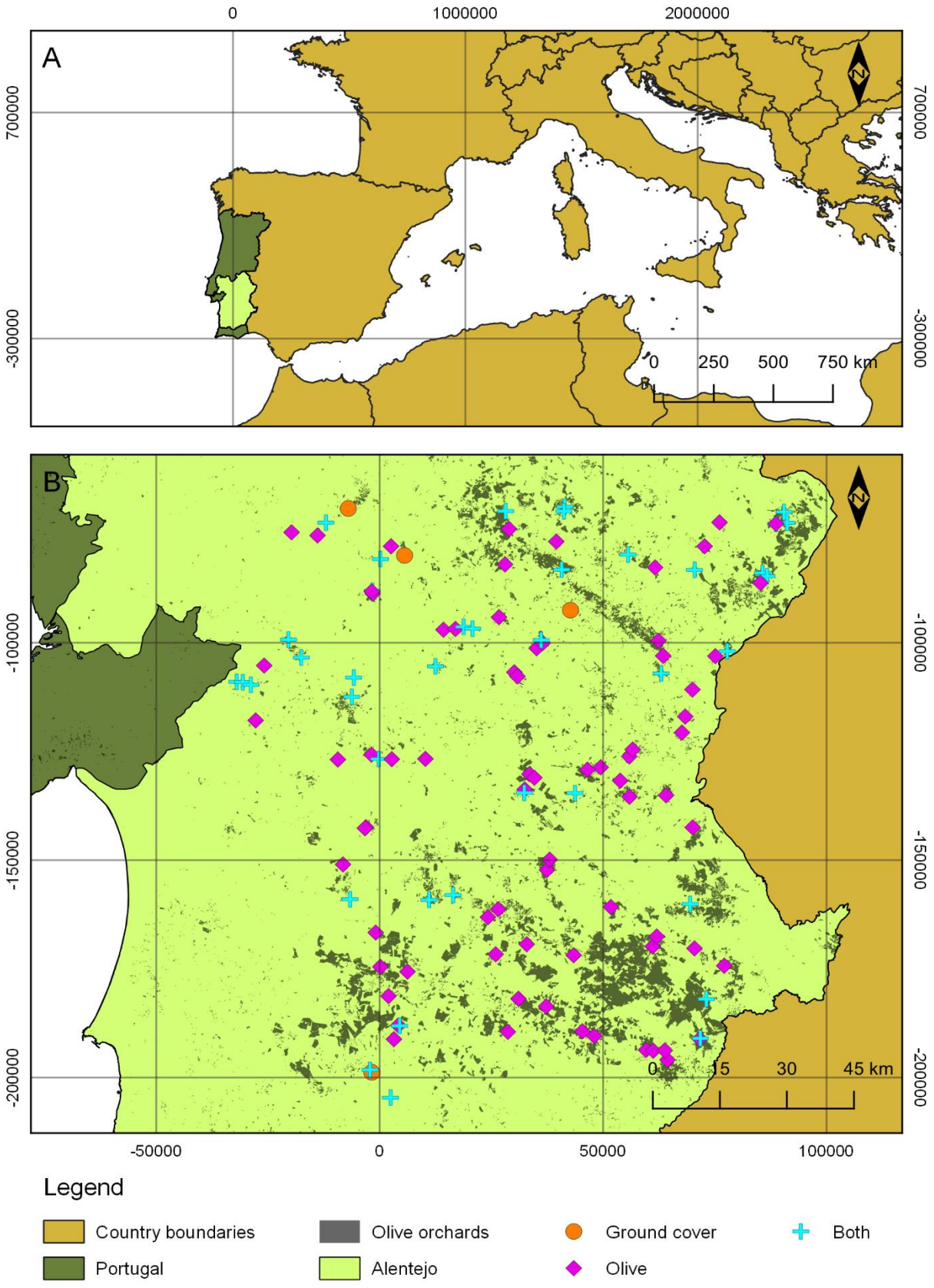

Figure 1: Alentejo localization (regarding other Portuguese regions and within the Mediterranean basin) map and data collection. (A) Map with the Alentejo (lighter green) localization within Mediterranean basin. (B) Samples location within Alentejo. Orange dots represent exclusive ground cover samplings; Pink diamonds represent exclusive olive canopy samplings; Blue crosses represent sampling of both the ground cover and olive canopy in that spot.

2.3. Morphological identification

The first step was the separation into higher taxonomic groups such as Formicidae, Araneae, Heteroptera, Neuroptera, etc. After this step, the individuals were grouped according to their morphology. Further sorting took place using a binocular magnifying glass model Olympus S2X7 and the appropriate identification keys were used within each group to reach species level identification:

- For the Neuroptera, Monserrat (2016) and Diaz-Aranda (1995) were used for the taxonomical identification to the species level.
- In the case of the Coccinellid group I used the keys by Hodek (1973), Raimundo (1986), Hackston (2012) and Bienkowski (2018) to identify to the species level.
- The family Formicidae was identified until the species using Collingwood (1998) and Lebas (2017).
- Heteroptera were first separated into families using Chinery (1977) and Mata (2013). Then, using their diet as criteria for further taxonomical identification since the specimens of interest for this thesis are predators, some families were further identified to genera and a few, to species level, using Wagner (1964), Pericart (1972), Pericart (1987), Schwartz (2008), Tatarnic (2012), Mata (2013) and Goula (2018).
- Pseudoscorpionida order was identified until the genera level using Buddle (2010), Harvey (2011) and Lissner (2014).
- Opiliones present in the sample were identified until the genera level using Hillyard (1989), Jones (1990), Oger (2014-present) and Richards (2017).
- The last group with interest for this work was the Araneae and were identified for the most part until the genera level. Some were possible to identify until the species and some were impossible to identify and therefore were assigned a morphotype and a code name. The identified individuals were identified using Jones (1990) and Barrientos (2003).

2.4. Photographic registry of specimens

All the insects which were identified to the genera or species level, were photographed using Zeiss Stereo Lumar V.12 coupled with a camera Axiocam 503 Color. The photos were taken using a z-stack program which took many photos in the z-axis. After that the photos were joined generating an unique photo using the extensive focus. In other words, the photos of the insects present in this thesis are not true photos but a combination of a stack of many.

2.5. Molecular identification

For DNA extraction the NZY Tissue gDNA Isolation Kit was used. Following the protocol described by the manufacture. It was used the complete individual for the DNA extraction.

The primer pair LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') of Former et al (1994) was subsequently used to amplify a 658 bp fragment of the COI gene. PCR was carried out a thermocycler with a final volume of 12.5 µL containing 0.25 µL dNTP (2 mM), 1.25 µL 10× Taq buffer, 0.25 µL each primer (10 mM), 0.7 µL $MgCl_2$ (50 mM), 0.05 U/mL Taq DNA polymerase, 1 µL of the extracted DNA (10-20 ng), and ultrapure water. These quantities correspond with the quantity needed for one sample. To the 24µL of used mix, was added 1µL of DNA. The PCR thermal regime consisted of one cycle of 4 min at 94 ºC; 30 sec at 94 °C, 30 sec at 50 °C, 1 min at 72°C and 10 min at 72 °C; during 35 cycles. In the PCR optimization process, we observed that some samples needed a dilution for correct amplification. Several dilutions were tested to reach the following: A2 and A3 samples were diluted to 1:10 (1µL of DNA and 9µL of distilled water) and A11 to 1:1000 dilution (1µL of DNA and 999µL of distilled water).

The F9, F10, A2, A3, A4, A9, A10 and A11 samples were amplified using a lower annealing temperature of 45°C instead of 50°C. The PCR products were observed in 1% agarose gel.
The amplified products were purified with the NZY Tissue gDNA Purification Kit, following the protocol described by the manufacturer. The products were sequenced by Eurofins Scientific.

2.6. Proof of principle of predation

The DNA extraction was also performed using the NZY Tissue gDNA Isolation Kit, following their protocol. It was used the complete individuals for the DNA extraction.
The primers SBo1-F (5′CAG TAG TAC TAA CAG CCC TAC T 3′), SBo2-F (5′TTA GCA GGT ATC TCC TCA ATC 3′) and SBo1-R (5′CTG GGT CGA AAA AGG AAG TAT′3) of Rejili (2016) were selected as *B. oleae* specific primers. These primers were used in different PCR mixes (one of SBo1-F and SBo1-R and other with SBo2-F and SBo1-R) but with the same quantities and concentrations. Using the pair SBo1-F/SBo1-R will result in a fragment 108 bp and one of 214 bp with SBo2-F/SBo1-R. Each PCR contained 15.8µL of distilled water, 5.0µL of Buffer, 1.5µL of Mg^{2+} (50 mM), 0.5µL of dNTPs (2 mM) (they were already mix), 0.5µL of each primer (forward and reverse) (10 mM) and 0.25µL of Taq polymerase. These quantities correspond with the quantity needed for one sample. To the 24µL of used mix, 1µL of DNA in the Mix 1 was added. In Mix 2, it was used 23µL of the mix and 2µL of DNA and Mix 3 had the same quantities of Mix 1 (24µL mix + 1µL DNA) but it switched the forward primer of SBo1-F to SBo2-F. The PCR thermal regime consisted of one cycle of 4 min at 94 °C; 30 sec at 94 °C, 30 sec at 50 °C, 1 min at 72°C and 10 min at 72 °C; during 35 cycles. After some trials the temperature of annealing was changed from 50°C to 48°C. For the Araneae samples, it was used a gradient PCR where the annealing temperature varied from 48°C to 58°C.
The amplified products were purified with the NZY Tissue gDNA Purification Kit, following the protocol described by the manufacture. The products were sequenced by Eurofins Scientific.

2.7. Data analyses

The maps created for this dissertation were made using QGIS (3.4.9 version) and ARCGIS.
The Ecological indexes were calculated using the R programme (3.4.2 version) with the "Vegan" package and the other calculus like dominance and frequency and total abundance were performed on Microsoft Excel (the one used in this work was 2016 version).
The measures defined for the species were frequency (relative) calculated as the number of presences of a given species divided by the number of samples of a strata spot (olive canopy or ground cover), total abundance calculated as the number of species individuals per strata spot and dominance calculated as the total abundance of a species divided by the sum of all the total abundance of all the species of that order and then multiplied by 100 (to give more perceivable numbers).
The ecological indexes calculated in this work were global abundance, richness, inverse Simpson index and Shannon index. The global abundance was calculated as the number of specimens per strata sample. Richness was calculated as the number of species per strata sample. The Simpson index measures the probability that two individuals randomly selected from a sample will belong to the same species (Simpson's index is represented with a D) (Krebs, 1989). This index can be expressed in other forms such as Simpson's Diversity (1-D) or Simpson's Reciprocal (1/D). The last version was the one used in this work. Lastly the Shannon index (H') tests how uniform or homogeneous the community (the species) is numerically in each strata sample (Krebs, 1989).

3. Results

3.1. Morphological identification

After the sorting, 203 morphospecies were identified in the sampling of putative predators, 26 non predators and 177 predators and generalists. The non-predator morphospecies were mainly from the Heteroptera taxa (20 species plus nymphs and unidentified morphotypes), 1 Coleoptera species and 5 Hymenoptera species (Table 1) (some of the non-predator morphospecies identified can be observed in Figure 2). In the case of the predators identified, 122 are Aranea, 8 are Coleoptera, 8 are Heteroptera, 25 are Hymenoptera, 1 is Mantodea, 4 are Neuroptera, 4 are Opiliones and 5 are Pseudoscorpiones (Table 2 and Table 3).

Table 1: List of all non-predator species identified.

ORDER	FAMILY	PUTATIVE SPECIES	N
Coleoptera	Coccinellidae	*Subcoccinella vigintiquattuorpunctata*	1
Heteroptera	Alydidae	*Camptopus lateralis*	2
		Micrelytra fossularum	5
		Nemausus simplex	6
	Blissidae	*Ischnodemus* sp.	1
	Coreidae	*Centrocoris variegatus*	1
		Gonocerus acuteangulatus	1
	Lygaeidae	*Nysius* sp.	19
		Oxycarenus lavaterae	434
		Spilostethus pandurus	3
	Miridae	*Apolygus spinolae*	33
		Calocoris roseomaculatus	1
		Lygus sp.	1
		Macrolophus melanotoma	1
		Macrolophus pygmaeus	10
		Trigonotylus sp.	1
	Pentatomidae	*Carpocoris melanocerus*	4
		Nezara viridula	3
	Pyrrhocoridae	*Pyrrhocoris apterus*	1
	Rhyparochromidae	*Plinthisus* sp.	2
	Thaumastocoridae	*Thaumastocoris peregrinus*	2
	-	Heteroptera morphotypes*	5
	-	Heteroptera nymphs*	296
Hymenoptera	Formicidae	*Cardiocondyla batesii*	2
		Messor barbarus	47
		Messor capitatus	15
		Messor structor	153
		Tetramorium chefteki	1

*In the case of the Heteroptera morphotypes and nymphs there might have some predators' species in there however there were times constraints to separate them.

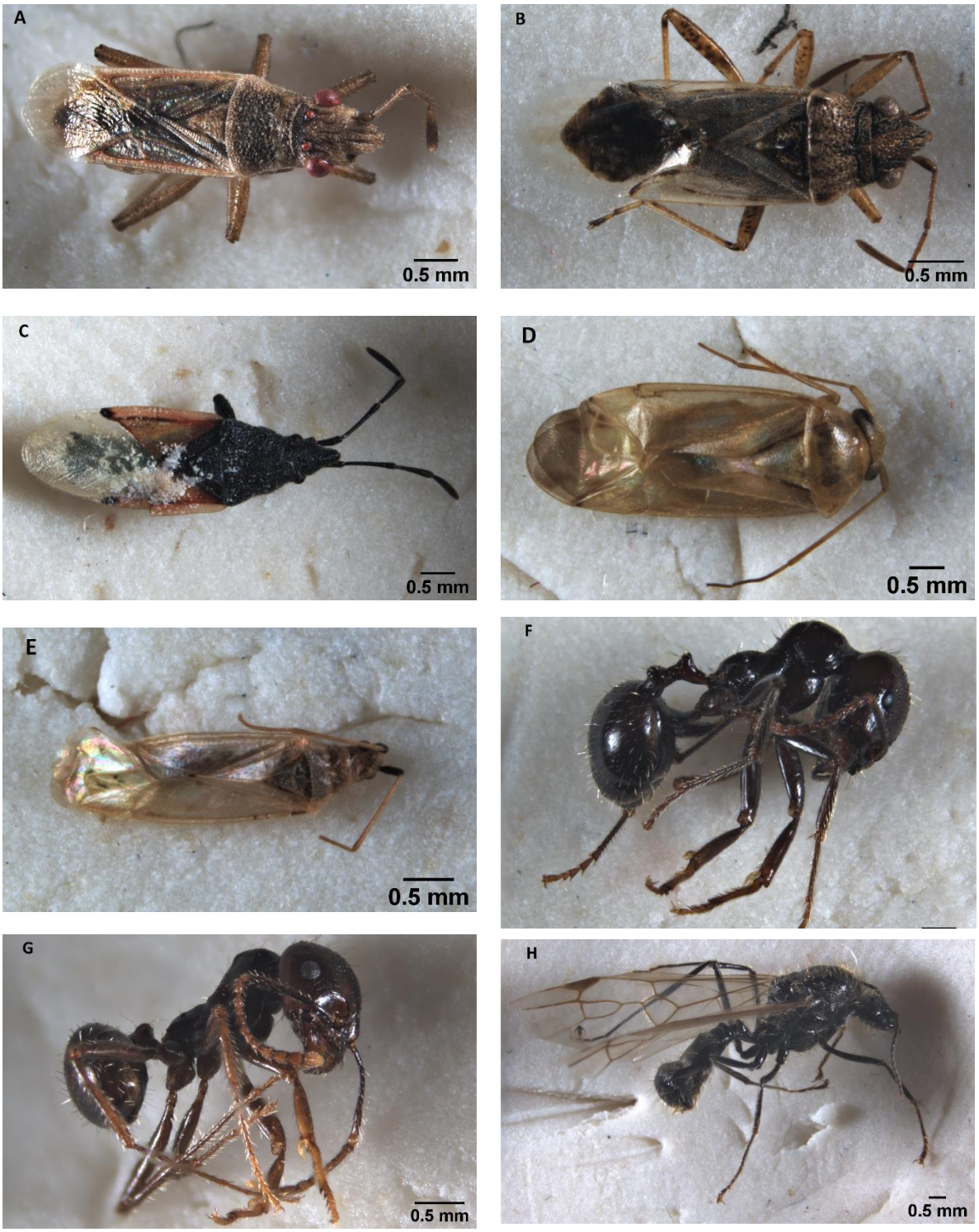

Figure 2: Photos of the non-predator species with more individuals. (A) *Nemausus simplex*. (B) *Nysius* sp. (C) *Oxycarenus lavaterae* with a fungal growth. (D) *Apolygus spinolae*. (E) *Macrolophus pygmaeus*. (F) *Messor barbarus*. (G) *Messor capitatus.* (H) *Messor structor*.

Table 2: List of all predator species identified of the Class Arachnida, with their respective number of captured individuals (N), dominance and frequency. The dominance and frequency are over the total of samples (olive canopy + ground cover).

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
Aranea	Anyphaenidae	*Anyphaena sp.*	4	0.22	1.28
	Araneidae	*Araneus sp.*	10	0.58	3.85
		Cyclosa sp.	2	0.11	1.28
		Cyrtarachne ixoides (Simon)	1	0.05	0.64
		Cyrtophora sp.	11	0.60	3.21
		Gibbaranea sp.	1	0.05	0.64
		Hypsosinga albovittata (Westring)	2	0.11	1.28
		Larinia lineata (Lucas)	1	0.05	0.64
		Larininoides sp.	2	0.11	1.28
		Mangora acalypha (Walckenaer)	10	0.55	3.21
		Mangora sp.	1	0.05	0.64
		Neoscona sp.	2	0.11	1.28
		Singa sp.	2	0.11	1.28
		Zilla diodia (Walckenaer)	11	0.60	3.85
		Zilla sp.	1	0.05	0.64
		*Zygiella sp.*1	18	0.99	0.26
		*Zygiella sp.*2	1	0.05	0.64
	Clubionidae	*Clubiona sp.*1	22	1.21	10.90
		Clubiona sp.2	2	0.11	0.64
	Dictynidae	*Lathys humilis (Blackwall)*	1	0.05	0.64
		Mastigusa arietina (Thorell)	2	0.11	1.28
		Mastigusa sp.	2	0.11	1.28
		Nigma sp.1	43	2.36	12.82
		Nigma sp.2	1	0.05	0.64
	Gnaphosidae	*Berlandina* sp.	2	0.11	1.28
		Callilepis sp.	1	0.05	0.64
		Civizelotes sp.	1	0.05	0.64
		Drassodes sp.	11	0.60	7.05
		Gnaphosa sp.	1	0.05	0.64
		Haplodrassus sp.	2	0.11	1.28
		Leptodrassus sp.	5	0.27	3.21
		Parasyrisca sp.	2	0.11	1.28
		Scotophaeus sp.	1	0.05	0.64
		Setaphis sp.	1	0.05	0.64
		Zelotes sp.	1	0.05	0.64
	Linyphiidae	*Drapetisca socialis* (Sundevall)	2	0.11	1.28

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Frontinella sp.	2	0.11	1.28
		Frontinellina frutetorum (C. L. Koch)	6	0.33	0.64
		Leptyphantes sp.	3	0.16	1.92
		Linyphia sp.	5	0.27	3.21
		Microlinyphia sp.	2	0.11	1.28
		Neriene sp.	20	1.10	7.05
		Peponocranium sp.	1	0.05	0.64
		Poeciloneta sp.	18	0.99	5.77
	Lycosidae	*Hygrolycosa rubrofasciata* (Ohlert)	3	0.16	1.92
		Pardosa sp.	1	0.05	0.64
		Trabaea sp.	3	0.16	1.28
		Trochosa sp.	6	0.33	3.21
	Mimetidae	*Ero* sp.	1	0.05	0.64
	Miturgidae	*Cheiracanthium* sp.	7	0.38	3.85
	Oxyopidae	*Oxyopes lineatus* Latreille	2	0.11	1.28
		Oxyopes sp.1	23	1.26	12.18
		Oxyopes sp.2	2	0.11	1.28
		Oxyopes sp.3	1	0.05	0.64
	Philodromidae	*Philodromus emarginatus* (Schrank)	1	0.05	0.64
		Philodromus sp.1	97	5.32	37.82
		Philodromus sp.2	21	1.15	10.90
		Philodromus sp.3	1	0.05	0.64
		Philodromus sp.4	2	0.11	1.28
		Thanathus oblongiusculus (Lucas)	2	0.11	1.28
		Thanatus sp.	5	0.27	2.56
		Tibellus sp.	3	0.16	1.92
	Pisauridae	*Dolomedes fimbriatus* (Clerck)	2	0.11	1.28
		Pisaura mirabilis (Clerck)	1	0.05	0.64
	Salticidae	*Chalcoscirtus* sp.	4	0.22	1.92
		Cyrba algerina (Lucas)	2	0.11	1.28
		Cyrba sp.	1	0.05	0.64
		Dendryphantes sp.	1	0.05	0.64
		Euophrys frontalis (Walckenaer)	1	0.05	0.64
		Euophrys sp.	3	0.16	1.92
		Evarcha sp.	2	0.11	1.28
		Hasarius adansoni (Audouin)	1	0.05	0.64
		Heliophanus sp.1	17	0.93	9.62

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Heliophanus sp.2	1	0.05	0.64
		Icius hamatus (C. L. Koch)	1	0.05	0.64
		Leptorchestes sp.	3	0.16	1.92
		Marpissa sp.	1	0.05	0.64
		Neon sp.	12	0.66	5.13
		Pellenes sp.	1	0.05	0.64
		Phlegra sp.	1	0.05	0.64
		Pseudeuophrys vafra (Blackwall)	1	0.05	0.64
		Saitis barbipes (Simon)	2	0.11	1.28
		Salticus cingulatus (Panzer)	1	0.05	0.64
		Salticus sp.	2	0.11	1.28
	Selenopidae	*Selenops* sp.	2	0.11	1.28
	Sparassidae	*Olios* sp.	4	0.22	2.56
	Tetragnathidae	*Meta* sp.	6	0.33	1.92
		Metellina sp.	4	0.22	1.28
		Pachygnatha sp.	4	0.22	1.92
		Tetragnatha sp.1	51	2.80	7.05
		Tetragnatha sp.2	2	0.11	0.64
	Theridiidae	*Anelosimus* sp.	18	0.99	3.85
		Crustalina sp.	8	0.44	3.21
		Dipoena sp.	6	0.33	2.56
		Enoplognatha sp.	12	0.66	5.77
		Episinus sp.	6	0.33	1.92
		Euryopis sp.	3	0.16	1.28
		Neottiura bimaculata (Linnaeus)	1	0.05	0.64
		Neottiura sp.	1	0.05	0.64
		Robertus sp.	2	0.11	1.28
		Rugathodes sp.	3	0.16	1.28
		Steatoda sp.	1	0.05	0.64
		Theridion sp.1	16	0.88	10.26
		Theridion sp.2	4	0.22	2.56
		Theridion sp.3	2	0.11	0.64
		Theridion sp.4	1	0.05	0.64
	Theridiosomatidae	*Theridiosoma* sp.	9	0.49	5.13
	Thomisidae	*Coriarachne* sp.	4	0.22	1.28
		Diaea sp.1	2	0.11	1.28
		Diaea sp.2	1	0.05	0.64
		Monaeses sp.	4	0.22	2.56
		Ozyptila sp.	3	0.16	1.92
		Runcinia sp.	19	1.04	9.62
		Synema sp.	1	0,05	0.64
		Thomisus sp.1	34	1.87	12.82

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Thomisus sp.2	1	0.05	0.64
		Tmarus sp.	8	0.44	5.13
		Xysticus sp.	13	0.71	6.41
	Uloboridae	*Uloborus* sp.	1	0.05	0.64
	Zodariidae	*Amphiledorus* sp.	1	0.05	0.64
		Selamia sp.	4	0.22	1.92
Opiliones	-	Opiliones Morphotype sp.1	2	40.00	0.64
	Phalangiidae	*Dicranopalpus* sp.	1	20.00	0.64
		Odiellus sp.	1	20.00	0.64
	Sclerosomatidae	*Leiobunum* sp.	1	20.00	0.64
Pseudoscorpiones	Cheliferidae	*Hysterochelifer tuberculatus* (Lucas)	2	6.06	0.64
	Chernetidae	*Pselaphochernes* sp.	1	3.03	0.64
	Chthoniidae	*Chthonius* sp.	2	6.06	1.28
	Geogarypidae	*Geogarypus nigrimanus* (Simon)	19	57.58	2.56
		Geogarypus sp.	9	27.27	3.21

Table 3: List of all predator species identified of the Class Insecta, with their respective number of captured individuals (N), dominance and frequency. The dominance and frequency are over the total of samples (olive canopy + ground cover).

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
Coleoptera	Coccinelidae	*Clitostethus arcuatus* (Rossi)	1	1.69	0.64
		Coccinella septempunctata Linnaeus	1	1.69	0.64
		Hippodamia variegata (Goeze)	1	1.69	0.64
		Propylea quatuordecimpunctata (Linnaeus)	2	3.39	0.64
		Scymnus abietis (Paykull)	4	6.78	2.56
		Scymnus apetzi Mulsant	2	3.39	0.64
		Scymnus mediterraneus Iablokoff-Khnzorian	25	42.37	8.33
		Stethorus punctillum (Weise)	22	37.29	8.97

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
Heteroptera	Anthocoridae	*Acompocoris pygmaeus* (Fallén)	1	0.11	0.64
		Anthocoris nemorum (Linnaeus)	4	0.46	2.56
	Miridae	*Compsidolon* sp.	1	0.11	0.64
		Deraeocoris serenus (Douglas and Scott)	1	0.11	0.64
		Dicyphus annulatus (Wolff)	1	0.11	0.64
		Dimorphocoris sp.	2	0.23	0.64
		Orthotylus sp.	32	3.67	6.41
	Nabidae	*Nabis* sp.	2	0.23	1.28
Hymenoptera	Formicidae	*Aphaenogaster senilis* Mayr	1	0.10	0.64
		Camponotus aethiops (Latreille)	2	0.20	1.28
		Camponotus barbaricus Emery	1	0.10	0.64
		Camponotus cruentatus (Latreille)	5	0.50	1.28
		Camponotus foreli Emery	1	0.10	0.64
		Camponotus lateralis (Olivier)	45	4.49	19.87
		Camponotus piceus (Leach)	1	0.10	0.64
		Camponotus pilicornis (Roger)	1	0.10	0.64
		Camponotus ruber	1	0.10	0.64
		Camponotus sicheli Mayr	1	0.10	0.64
		Cardiocondyla sp.	4	0.40	0.64
		Crematogaster auberti Emery	20	2.00	4.49
		Crematogaster scutellaris (Olivier)	155	15.47	25.00
		Crematogaster sordidula (Nylander)	51	5.09	10.26
		Formica subrufa (Roger)	6	0.60	2.56
		Lasius alienus (Foerster)	3	0.30	0.64
		Lasius brunneus (Latreille)	22	2.20	3.21
		Plagiolepis pygmaea (Latreille)	306	30.54	32.05

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Plagiolepis schmitzi (Forel)	64	6.39	8.97
		Plagiolepis sp.	7	0.70	1.92
		Tapinoma sp.1 (*nigerrimum-simrothi* complex)	76	7.58	5.77
		Temnothorax sp.1 (*recedens* complex)	1	0.10	0.64
		Tetramorium caespitum (Linnaeus)	1	0.10	0.64
		Tetramorium semilaeve André	1	0.10	0.64
		Tetramorium sp.1 (*simillimum* complex)	1	0.10	0.64
Mantodea	Mantidae	*Ameles spallanzania* (Rossi)	1	100.00	0.64
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Stephens)	58	80.56	21.79
		Chrysoperla sp.	5	6.94	3.21
		Cunctochrysa baetica (Hölzel)	4	5.56	1.92
		Cunctochrysa sp.	5	6.94	2.56

3.2. Olive canopy species

3.2.1. General characterization of the olive samples

From the total predators and generalist's morphospecies identified (which were 177 morphospecies), 127 morphospecies were found in the olive canopy samples, being 69 of them present only in the olive canopy samples (not in ground cover samples) (Annex - Table A1). The taxa with more exclusive morphospecies identified was Araneae, with 52 morphospecies, followed by Hymenoptera with 16 morphospecies and Coleoptera with 7 morphospecies (some of the exclusive morphospecies found in the olive canopy can be observed in Figure 4). Morphospecies of the Mantodea and Pseudoscorpiones taxa were not found in the olive canopy samples. Also, there were 7 families which were exclusively found in the olive canopy samples, 4 of them are Araneae (Anyphaenidae, Mimetidae, Pisauridae and Sparassidae), 2 are Opiliones (Phalangiidae and Sclerosomatidae) and 1 is Heteroptera (Anthocoridae) (Figure 3).

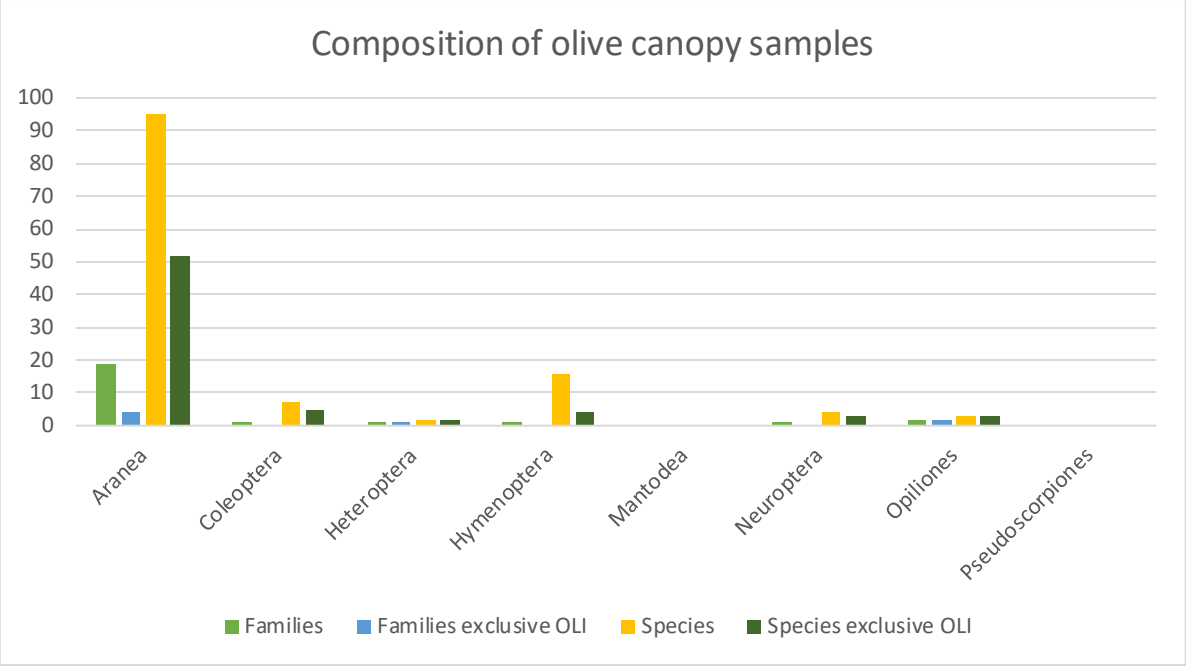

Figure 3: Composition of the olive canopy samples regarding the number of families and species of the taxa present (the numbers are the true values of species and families). The data is separated in relation with the non-exclusivity/exclusivity to the olive canopy.

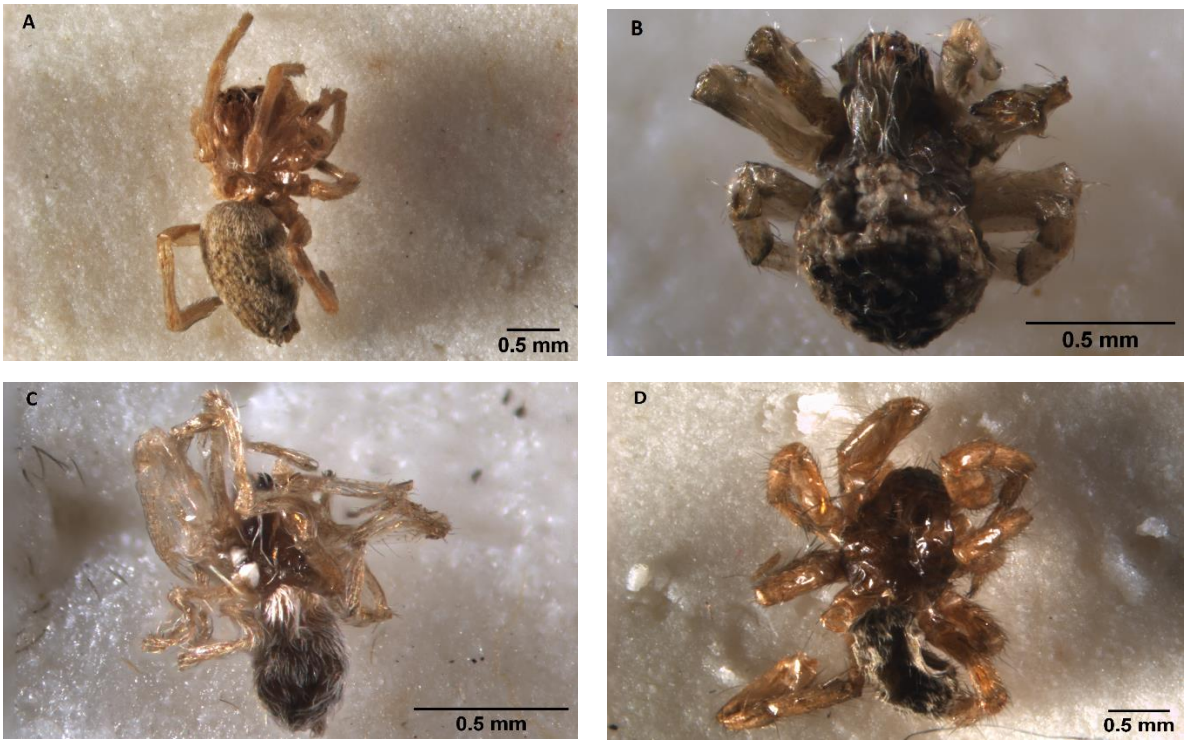


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Figure 4: Photos of some of the species that only appeared on the olive canopy samples. (A) *Anyphaena* sp. (B) *Zilla diodia*. (C) *Nigma* sp.1. (D) *Leptodrassus* sp. (E) *Peponocranium* sp. (F) *Ero* sp. (G) *Cyrba algerina*. (H) *Olios* sp. (I) *Propylea quatuordecimpunctata*. (J) *Scymnus apetzi*. (K) *Anthocoris nemorum*. (L) *Camponotus pilicornis*. (M) *Chrysoperla carnea*. (N) *Cunctochrysa baetica*. (O) *Dicranopalpus* sp. (P) *Leiobunum* sp.

3.2.2. Characterization of olive canopy species

For each morphospecies identified morphologically the frequency, the total abundance and dominance in the samples were calculated. The frequency and dominance are presented in Annex - Table A1. The morphospecies with higher frequency – 38.94% was *Philodromus* sp.1 (Order Araneae, Family Philodromidae). From all the morphospecies identified in olive canopy samples, 62 of them had the lowest frequency (0.89%). From that pool, 46 of them were Araneae, 4 Coleoptera, 1 Heteroptera, 8 Hymenoptera and 3 Opiliones. The mean frequency in the sampling pool was of 3.59% (Figure 5). The morphospecies with the highest total abundance – 129 - was *Crematogaster scutellaris* (Order Hymenoptera, Family Formicidae). The lowest total abundance - 1 – was present in 53 morphospecies of the olive canopy samples. From that 53 morphospecies with the lowest total abundance, 41 were Araneae, 2 Coleoptera, 1 Heteroptera, 6 Hymenoptera and 3 Opiliones. The mean total abundance of the olive samples was of 6.95 (Figure 6). In relation to dominance, the morphospecies with higher dominance was *Chrysoperla carnea* (Order Neuroptera, Family Chrysopidae) with 82.86%. The lowest dominance (0.89%) was present in 39 morphospecies of olive canopy samples, all of them of the Araneae Order. The mean dominance in olive canopy samples was 3.22% (Figure 7).

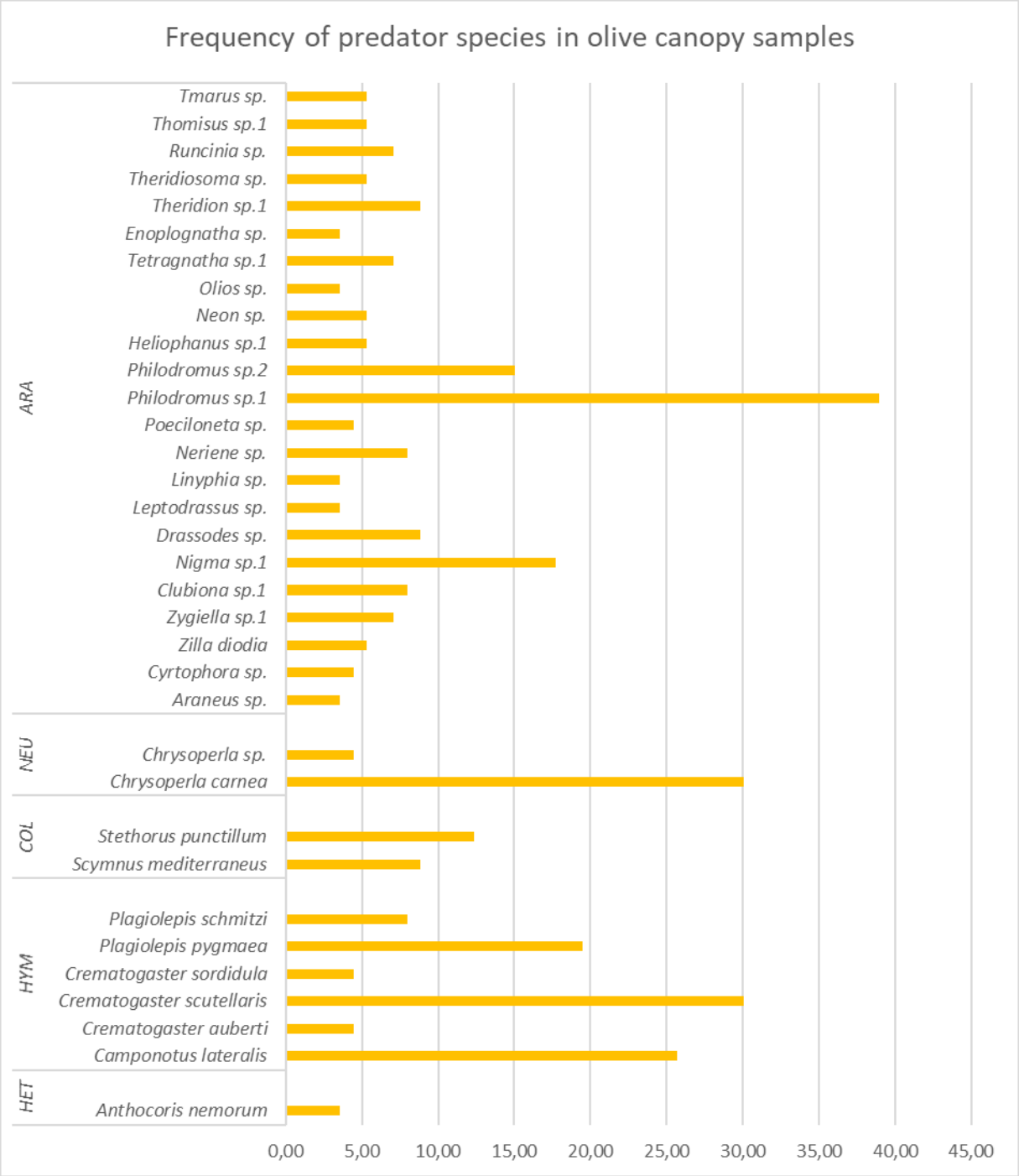

Figure 5: Frequency of varied predators in the olive canopy samples. The species present in this image correspond only with species with values similar and superior to the mean (Frequency mean=3.589). The Taxa are separated by the Order: ARA (Araneae), NEU (Neuroptera), COL (Coleoptera), HYM (Hymenoptera) and HET (Heteroptera). From bottom to top: Heteroptera, Hymenoptera, Coleoptera, Neuroptera and Araneae. *Philodromus* sp.1 has the highest frequency – 38.94%.

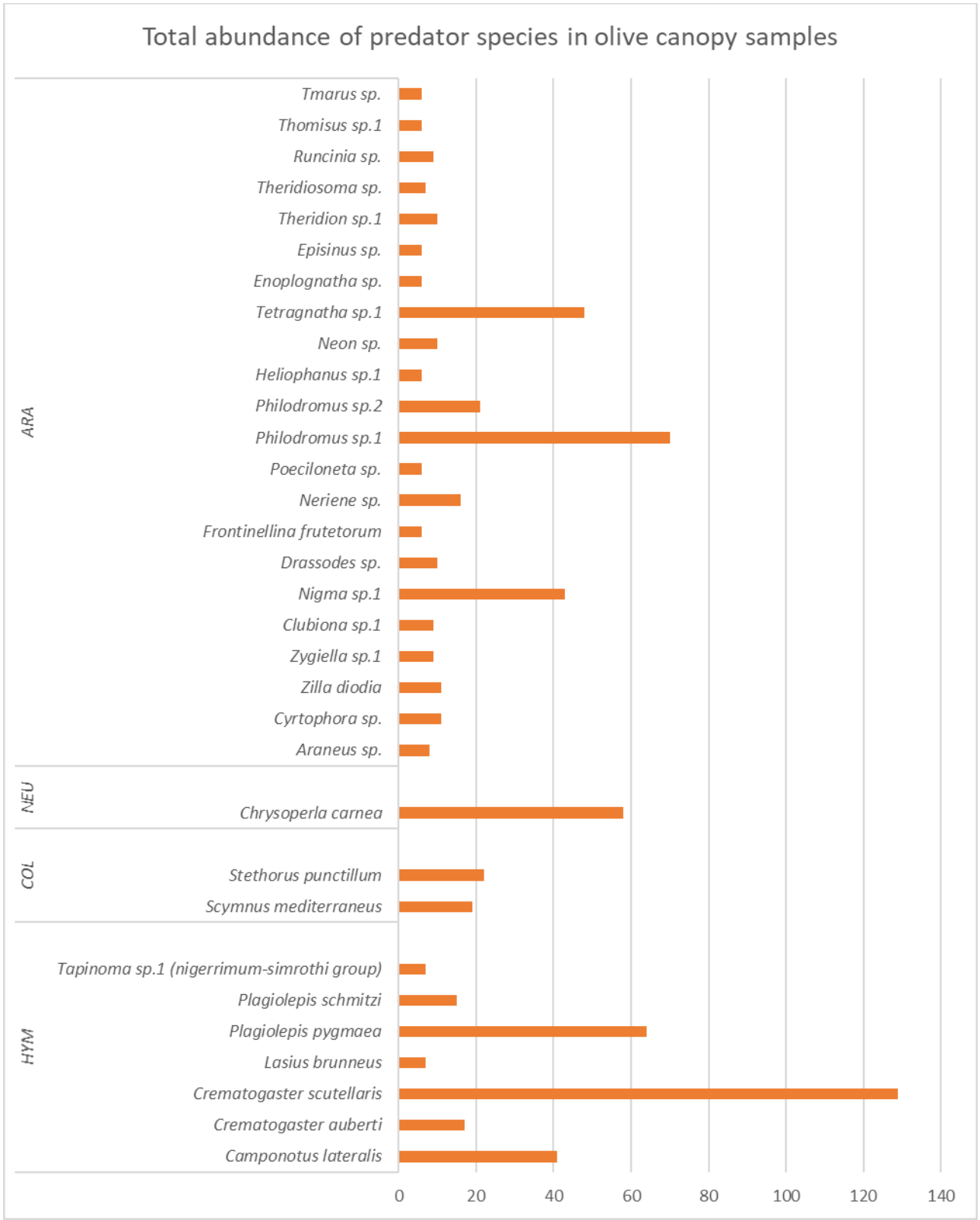

Figure 6: Total abundance of varied predators in the olive canopy samples. The species present in this image correspond only with species with values similar and superior to the mean (Total abundance mean=6.953). The Taxa are separated by the Order: ARA (Araneae), NEU (Neuroptera), COL (Coleoptera) and HYM (Hymenoptera). From bottom to top: Hymenoptera, Coleoptera, Neuroptera and Araneae. *Crematogaster scutellaris* has the highest abundance – 129.

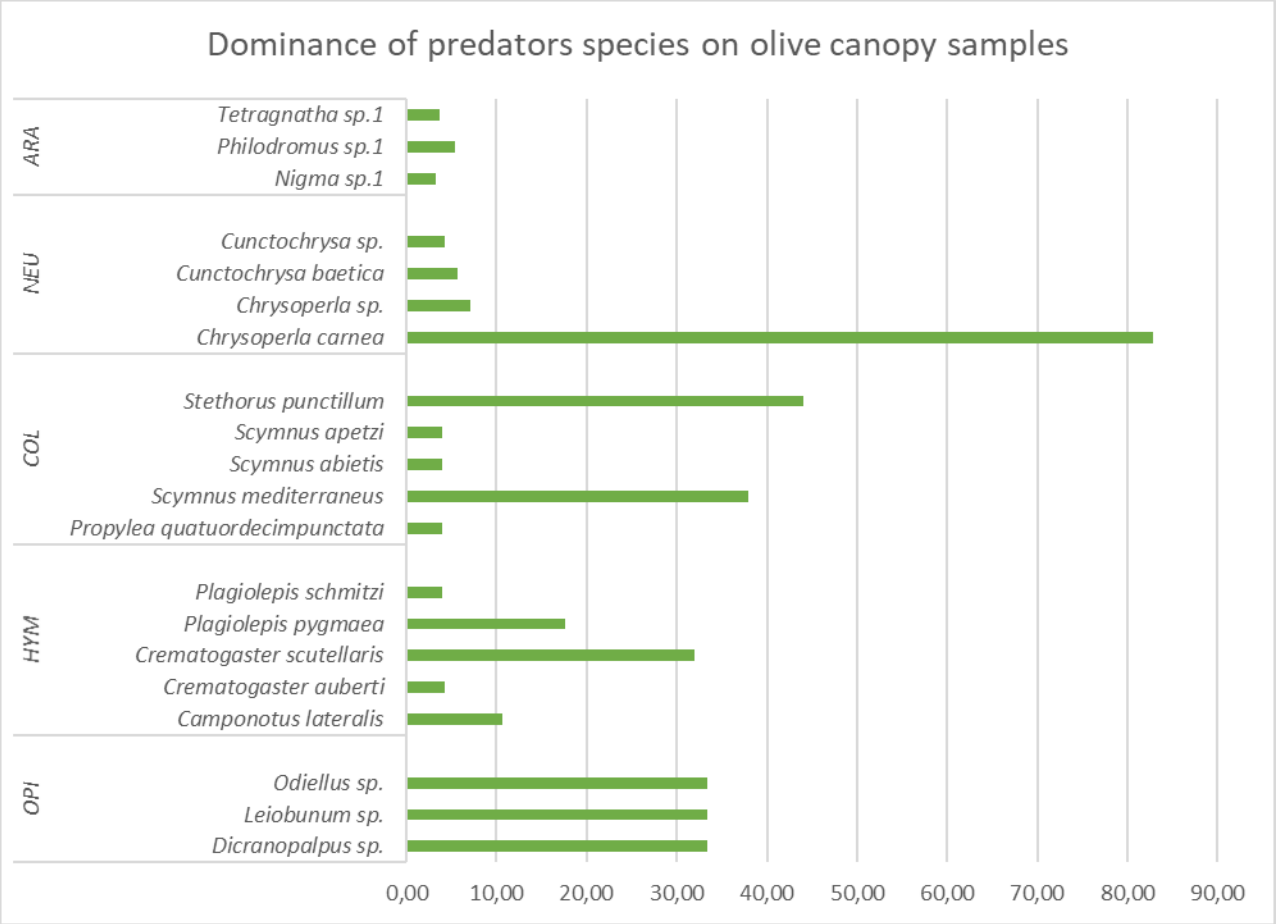

Figure 7: Dominance of varied predators in the olive canopy samples. The species present in this image correspond only with species with values similar and superior to the mean (mean=3.593). The Taxa are separated by the Order: ARA (Araneae), NEU (Neuroptera), COL (Coleoptera), HYM (Hymenoptera) and OPI (Opiliones). From bottom to top: Opiliones, Hymenoptera, Coleoptera, Neuroptera and Araneae. *Chrysoperla carnea* has the highest dominance – 82.86%.

3.2.3. Characterization of olive canopy samples

The global abundance (number of predator specimens) varied between 0 and 52 (Figure 8) with 11.5% of the samples showing a global abundance of 1. Overall, the sampling locations presented a low abundance, with an average of less than 8 specimens of predators per sample (mean = 7.96). The predators' species richness maximum was of 16 while the average was of 4.65, almost 4 times lower, and the mode was 4.00 (at 18 of 113 sampling locations; Figure 9). The Inverse Simpson Index (also referred as Reciprocal Simpson Index), ranged from 0 to 12.50, with a mean of 3.56 and a mode of 1.00 (Figure 10). The Shannon Index maximum value was of 2.58, with an average of 1.19. The mode was also the minimum observed value for this index, 0.00 (Figure 11). In each one of these biodiversity measures, only one sample location presented the highest value.

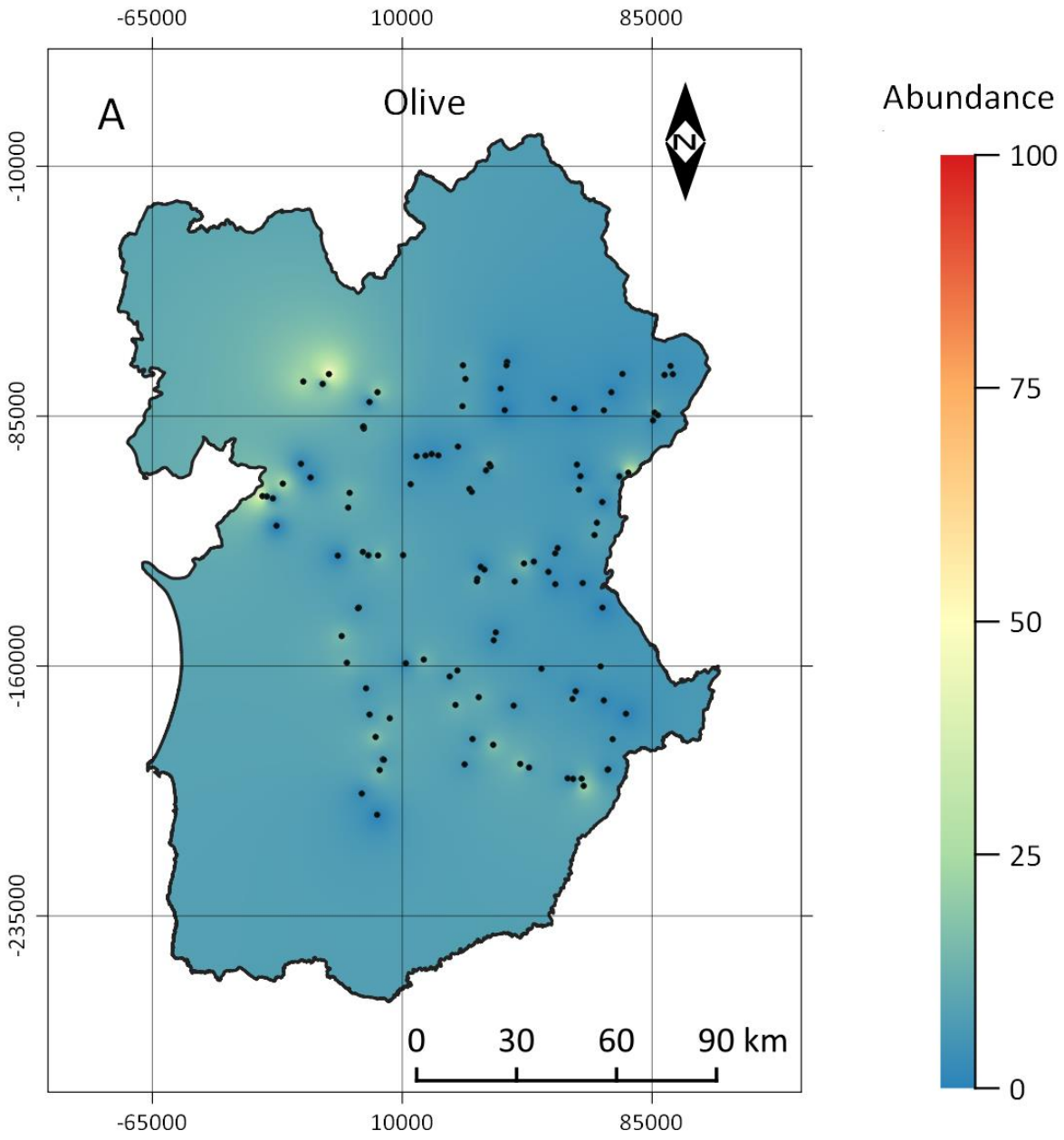

Figure 8: Map of global abundance of species identified in the olive canopy sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). Most spots have low abundance (mean = 7.96). The highest abundance found in the olive canopy samples was 52.00, of the sample point Q6 P113 OLI (Annex A – Table A5).

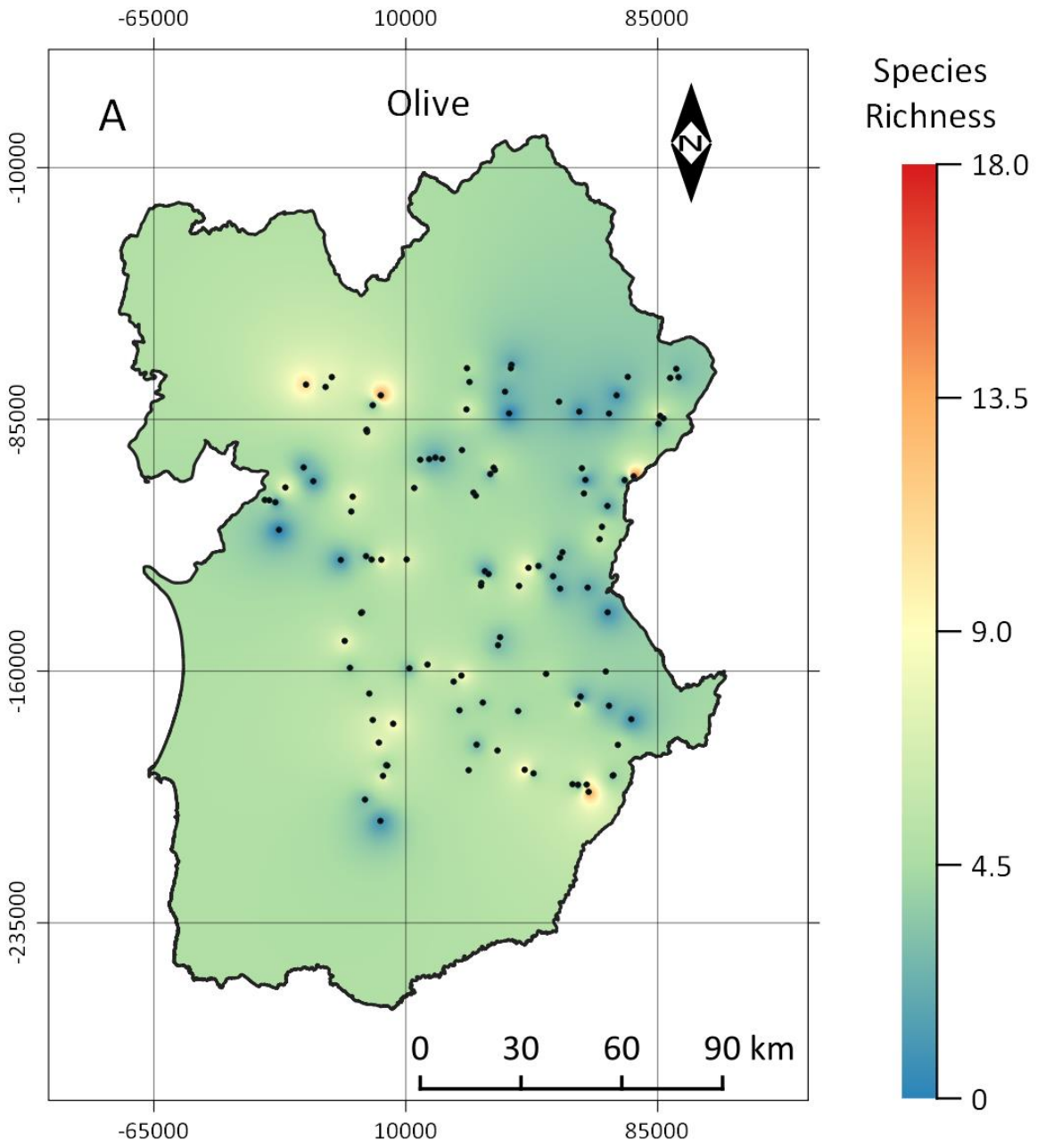

Figure 9: Map of global species richness identified in the olive canopy sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). Some sample points had very low species richness and the others had median levels of species richness (mean = 4.65). The sample point with the highest species richness was Q9 P142 OLI (Annex A – Table A5) – 16.00.

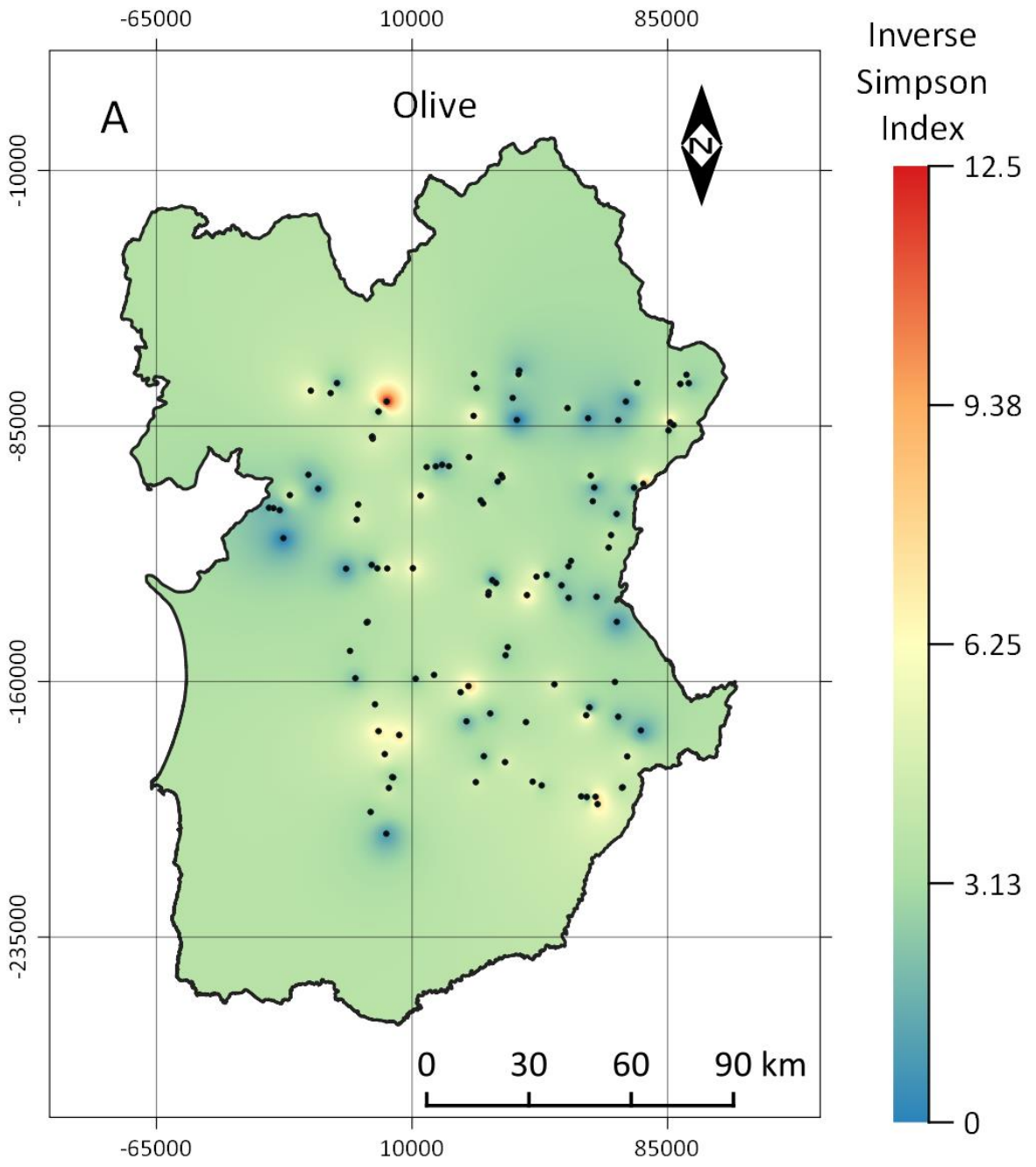

Figure 10: Map of global diversity index – inverse Simpson index - identified in the olive canopy sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). The sample with highest Inverse Simpson Index was Q1 P150 OLI (Annex A – Table A5) with 12.50 of value. The mean value of Inverse Simpson Index was 3.56.

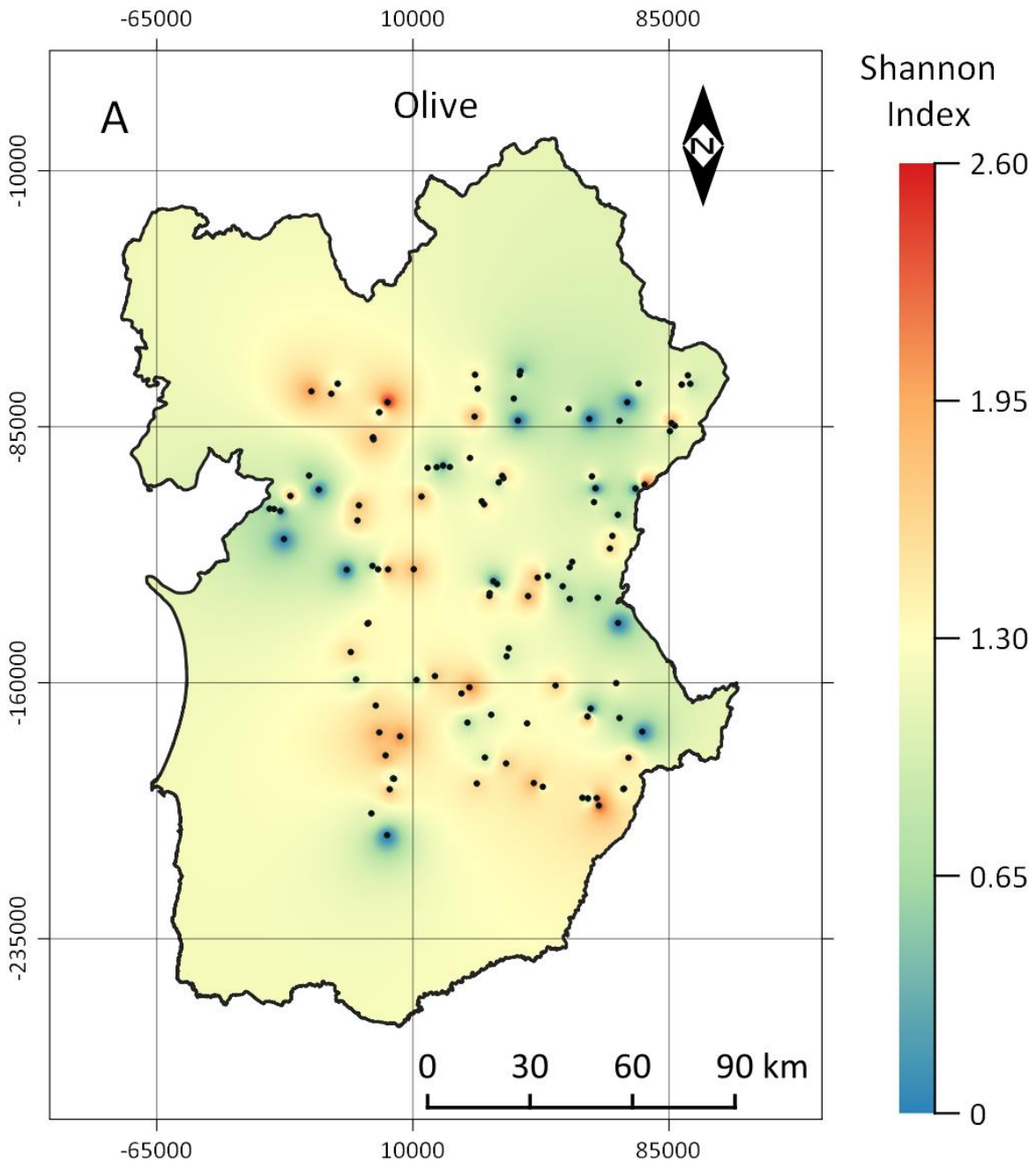

Figure 11: Map of global diversity index – Shannon index - identified in the olive canopy sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). The mean value among the samples for this index was 1.19. The sample point with the highest Shannon Index was Q1 P150 OLI (Annex A – Table A5) – 2.58.

3.3. Ground cover species

From the total predators and generalist's morphospecies identified (which were 177 morphospecies), 107 morphospecies were found in the ground cover samples, being 49 of them present only in the ground cover samples (not in olive canopy samples) (Annex A - Table A2). The taxa with more exclusive species identified was Araneae, with 26 morphospecies, followed by Hymenoptera with 9 morphospecies and Heteroptera with 6 morphospecies (some of the exclusive morphospecies found in

the ground cover can be observed in Figure 13). Every order identified in the totality of samples (canopy + ground cover), is present in ground cover samples. Also, there were 9 families which were exclusively found in the ground cover samples, 2 of them are Araneae (Selenopidae and Uloboridae), 2 are Heteroptera (Miridae and Nabidae), 1 is Mantodea (Mantidae) and 4 are Pseudoscorpiones (Cheliferidae, Chernetidae, Chthoniidae and Geogarypidae) (Figure 12).

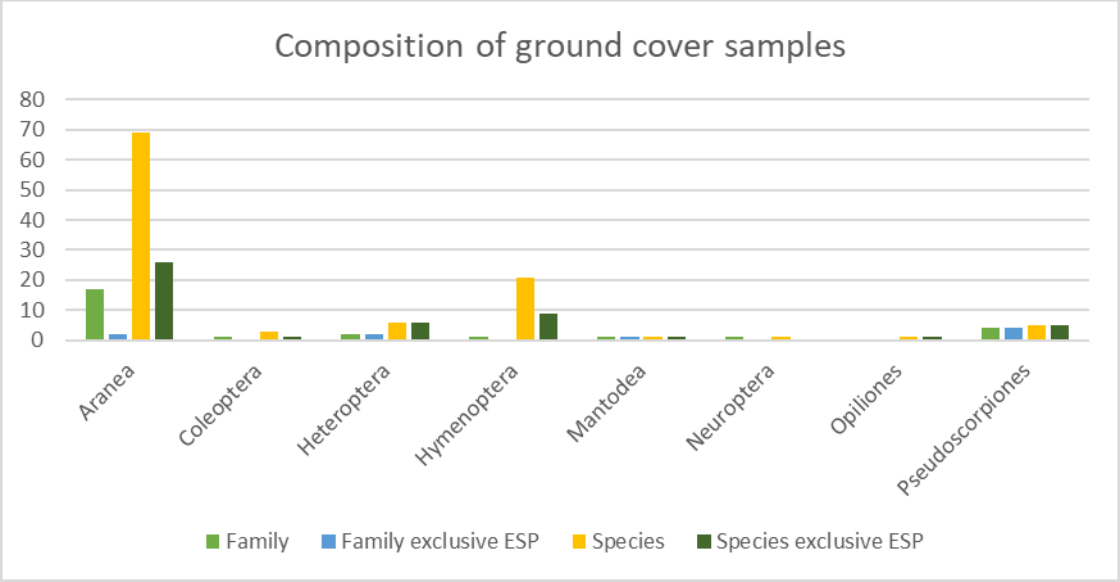

Figure 12: Composition of the ground cover samples regarding the number of families and species of the taxa present (the numbers are the true values of species and families). The data is separated in relation with the non-exclusivity/exclusivity to the ground cover.


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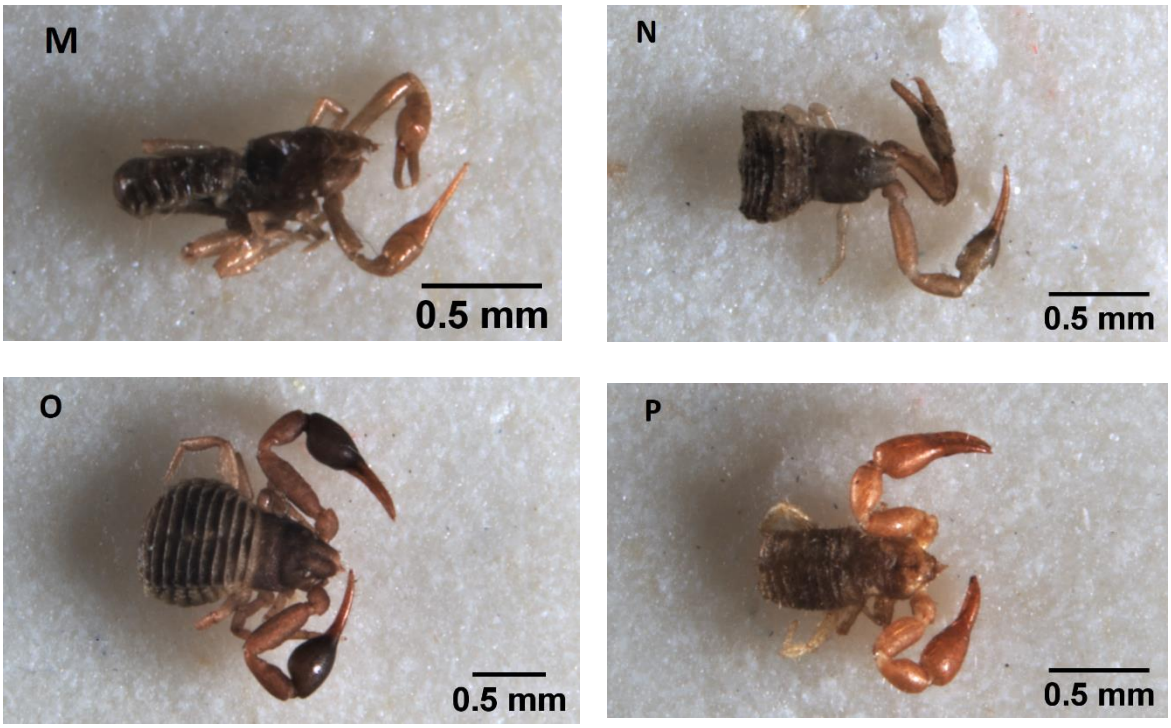

Figure 13: Photos of some of the species that only appeared on the ground cover samples. (A) *Trochosa* sp. (B) *Euophrys frontali*. (C) *Selenops* sp. (D) *Euryopis* sp. (E) *Coriarachne* sp. (F) *Uloborus* sp. (G) *Selamia sp.* (H) *Nabis* sp. (I) *Orthotylus sp.* (J) *Aphaenogaster senilis* (K*) Cardiocondyla* sp. (L) *Ameles spallanzania*. (M) *Chthonius sp.* (N) *Hysterochelifer tuberculatus*. (O) *Geogarypus nigrimanus*. (P) *Pselaphochernes sp.*

3.3.1. Characterization of ground cover species

The ground cover species' frequency and dominance are presented in Annex A - Table A2. The morphospecies with higher frequency – 55.81% is *Plagiolepis pygmaea* (Order Hymenoptera, Family Formicidae). From all the morphospecies identified in ground cover samples, 55 of them had the lowest frequency (2.33%). From that pool, 33 of them were Araneae, 1 Coleoptera, 4 Heteroptera, 12 Hymenoptera, 1 Mantodea, 1 Neuroptera, 1 Opiliones and 2 Pseudoscorpiones. The mean frequency in the sampling pool was of 6.59% (Figure 14). The morphospecies with the highest total abundance – 230 - was *Plagiolepis pygmaea* (Order Hymenoptera, Family Formicidae). The lowest total abundance – 1 – was present in 46 morphospecies of the ground cover samples. From that 46 morphospecies with the lowest total abundance, 32 were Araneae, 1 Coleoptera, 3 Heteroptera, 8 Hymenoptera, 1 Mantodea and 1 Pseudoscorpiones. The mean total abundance of the ground cover samples was of 8.00 (Figure 15). In relation to dominance, the morphospecies with higher dominance were *Ameles spallanzania* (Order Mantodea, Family Mantidae), *Cunctochrysa* sp. (Order Neuroptera, Family Chrysopidae) and Opiliones Morphotype sp.1 with 100.00%. The lowest dominance (0.17%) was present in 8 morphospecies of ground cover samples, all of them of the Hymenoptera Order. The mean dominance in ground cover samples was 6.02% (Figure 16).

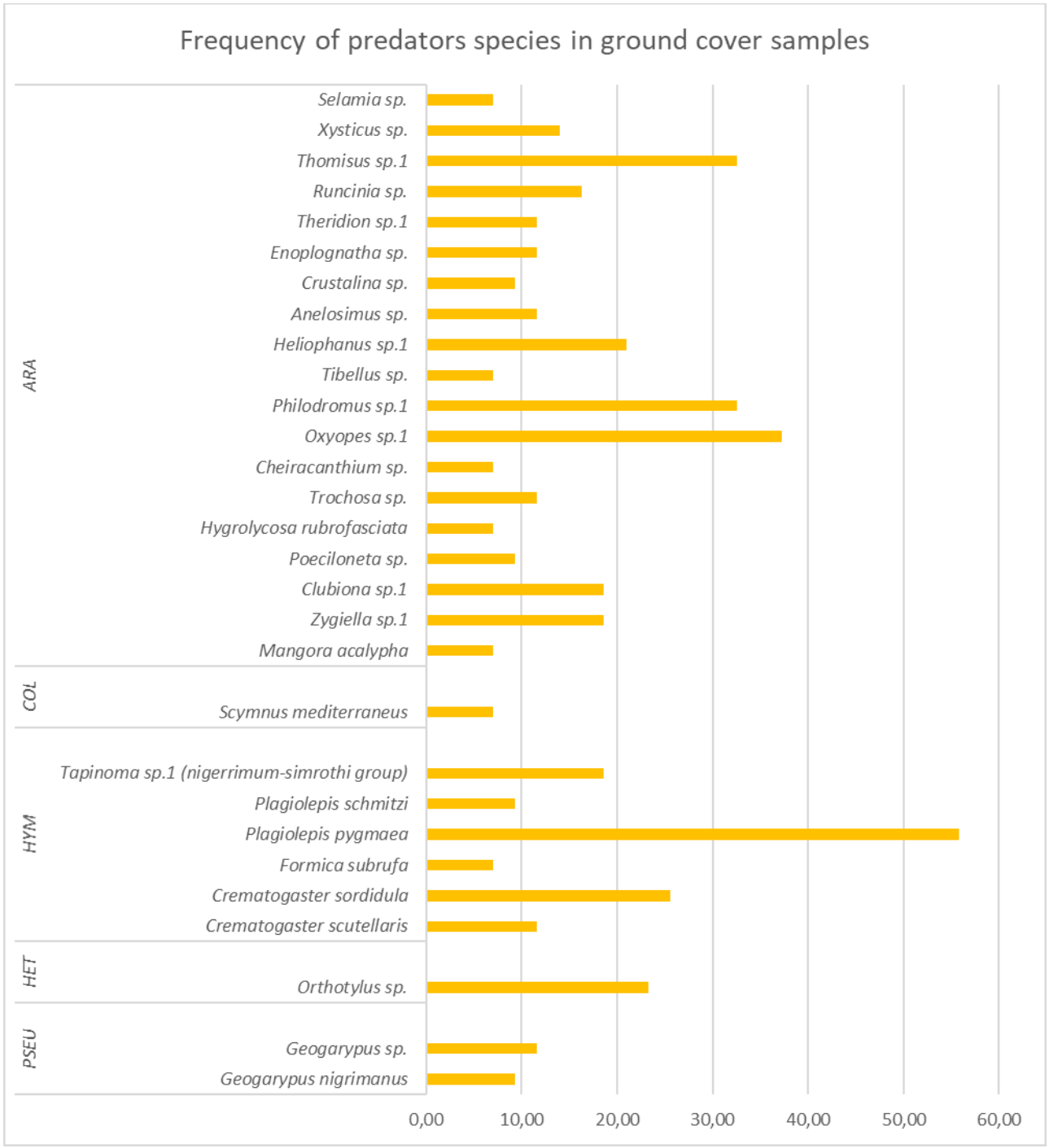

Figure 14: Frequency of varied predator species in ground cover samples. The species present in this image correspond only with species with values similar and superior to the mean (Frequency mean=6.586). The Taxa are separated by the Order: ARA (Araneae), COL (Coleoptera), HYM (Hymenoptera), HET (Heteroptera) and PSEU (Pseudoscorpiones). From bottom to top: Pseudoscorpiones, Heteroptera, Hymenoptera, Coleoptera and Araneae. *Plagiolepis pygmaea* was the species with the highest frequency – 55.81%.

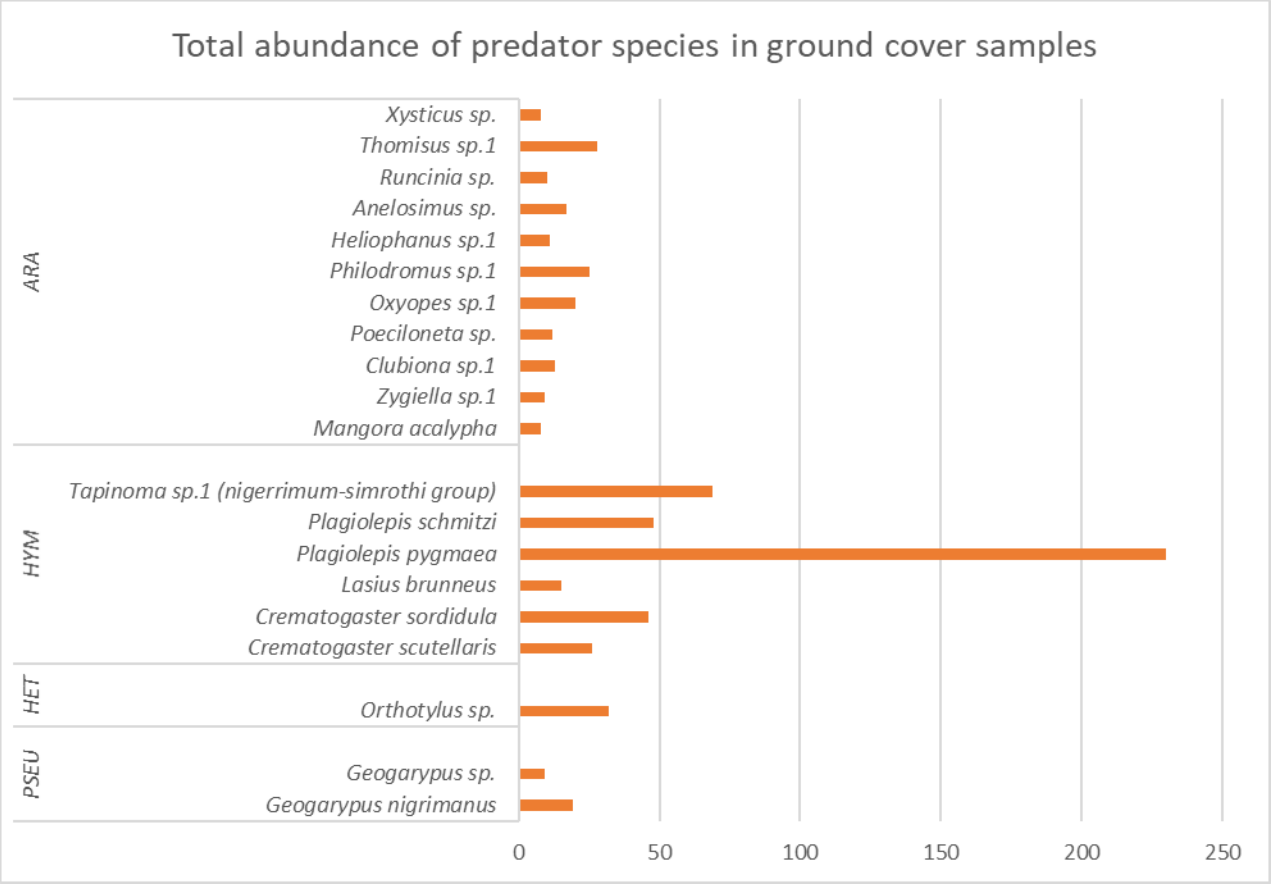

Figure 15: Total abundance of varied predator species in ground cover samples. The species present in this image correspond only with species with values similar and superior to the mean (Total abundance mean=7.757). The Taxa are separated by the Order: ARA (Araneae), HYM (Hymenoptera), HET (Heteroptera) and PSEU (Pseudoscorpiones). From bottom to top: Pseudoscorpiones, Heteroptera, Hymenoptera and Araneae. *Plagiolepis pygmaea* was the species with the highest abundance – 230.

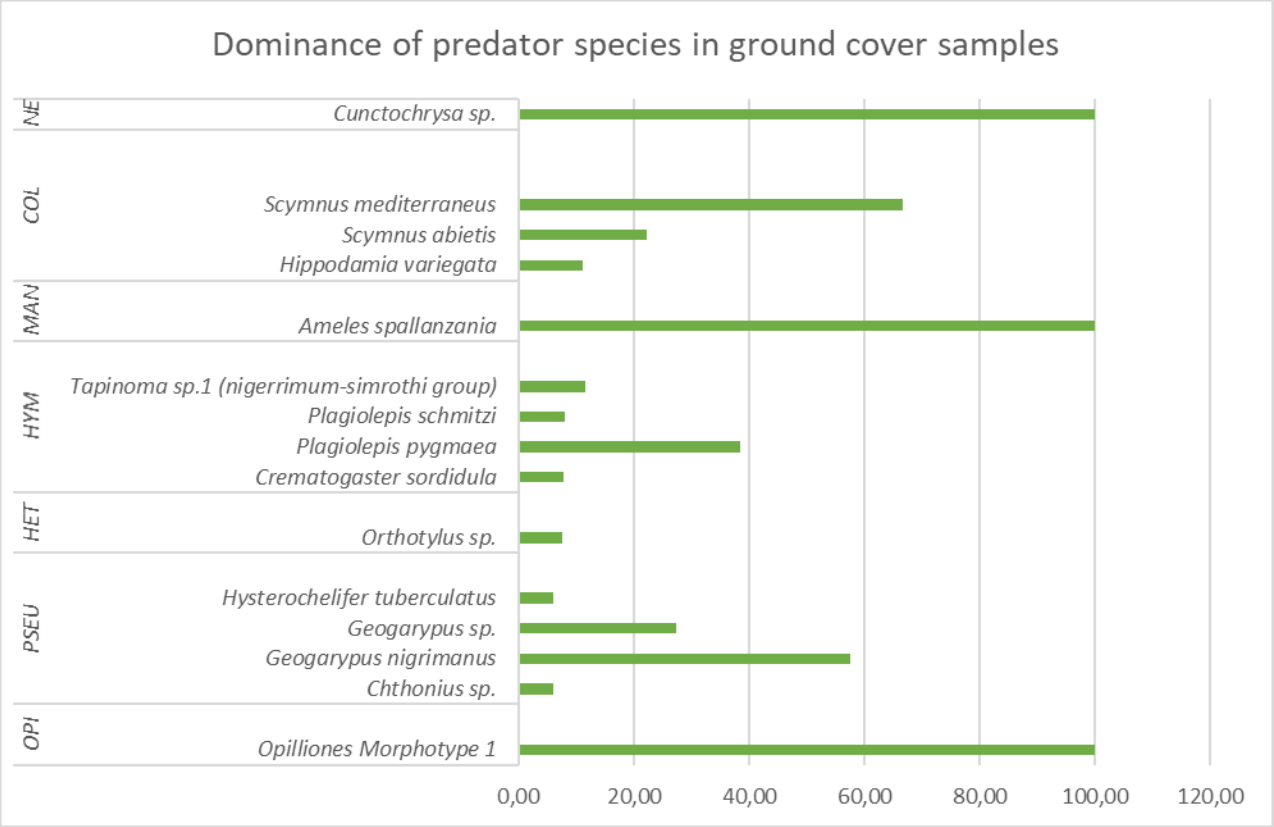

Figure 16: Dominance of varied predator species in ground cover samples. The species present in this image correspond only with species with values similar and superior to the mean (mean=6.015). The Taxa are separated by the Order: NE (Neuroptera), COL (Coleoptera), MAN (Mantodea), HYM (Hymenoptera), HET (Heteroptera), PSEU (Pseudoscorpiones) and OPI (Opiliones). The species with the highest dominance were *Cunctochrysa* sp., *Ameles spallanzania* and Opiliones Morphotype 1, all with 100.00%.

3.3.2. Characterization of ground cover samples

The global abundance varied between 0 and 98 (Figure 17), with only 2 sampling locations having the lowest value. The mode for the global abundance was 11, with 11.6% of the sample locations presenting this value. The highest value of species richness was 18 and the lowest value was 0 (present in 2 sample spots). The mean species richness was 7.07 and the mode was 8.00 (almost half the highest species richness value), with 23.3% of the sampling points presenting this value (Figure 18). The Inverse Simpson Index ranged from 0 to 10.29, with a mean of 4.40 and a mode of 1.00, with 7% of the sample points presenting this value (Figure 19). The Shannon Index maximum value observed was 2.50, with an average of 1.48. The mode was also the minimum observed value for this index, 0.00, with 2.4% of the sampling locations presenting this value (Figure 20).

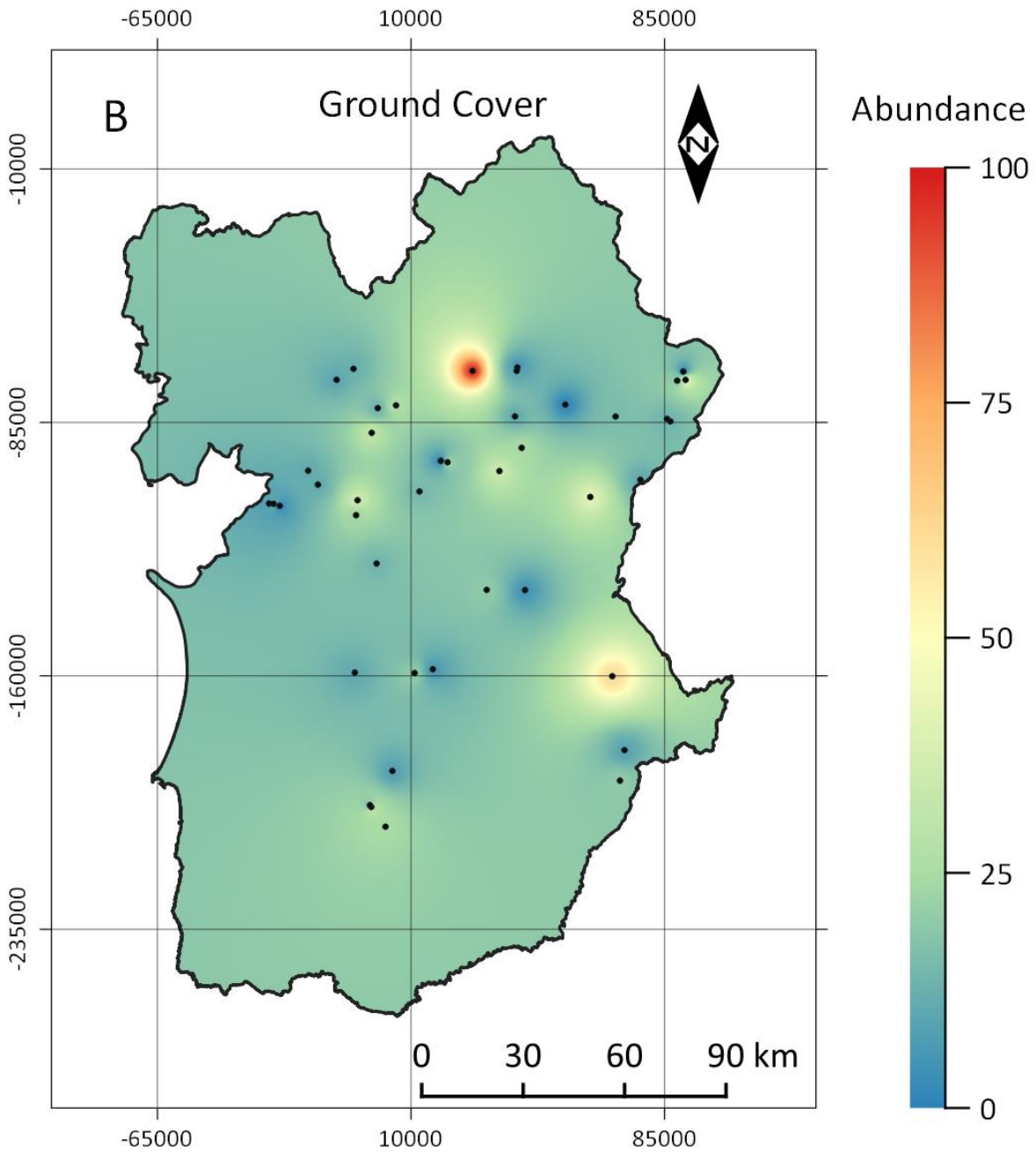

Figure 17: Map of global abundance of species identified in the ground cover sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). The abundance in the sample spots is very low (mean = 19.42) except for the point with highest abundance – Q2 P162 ESP (Annex A – Table A5) – with a value of 98.00.

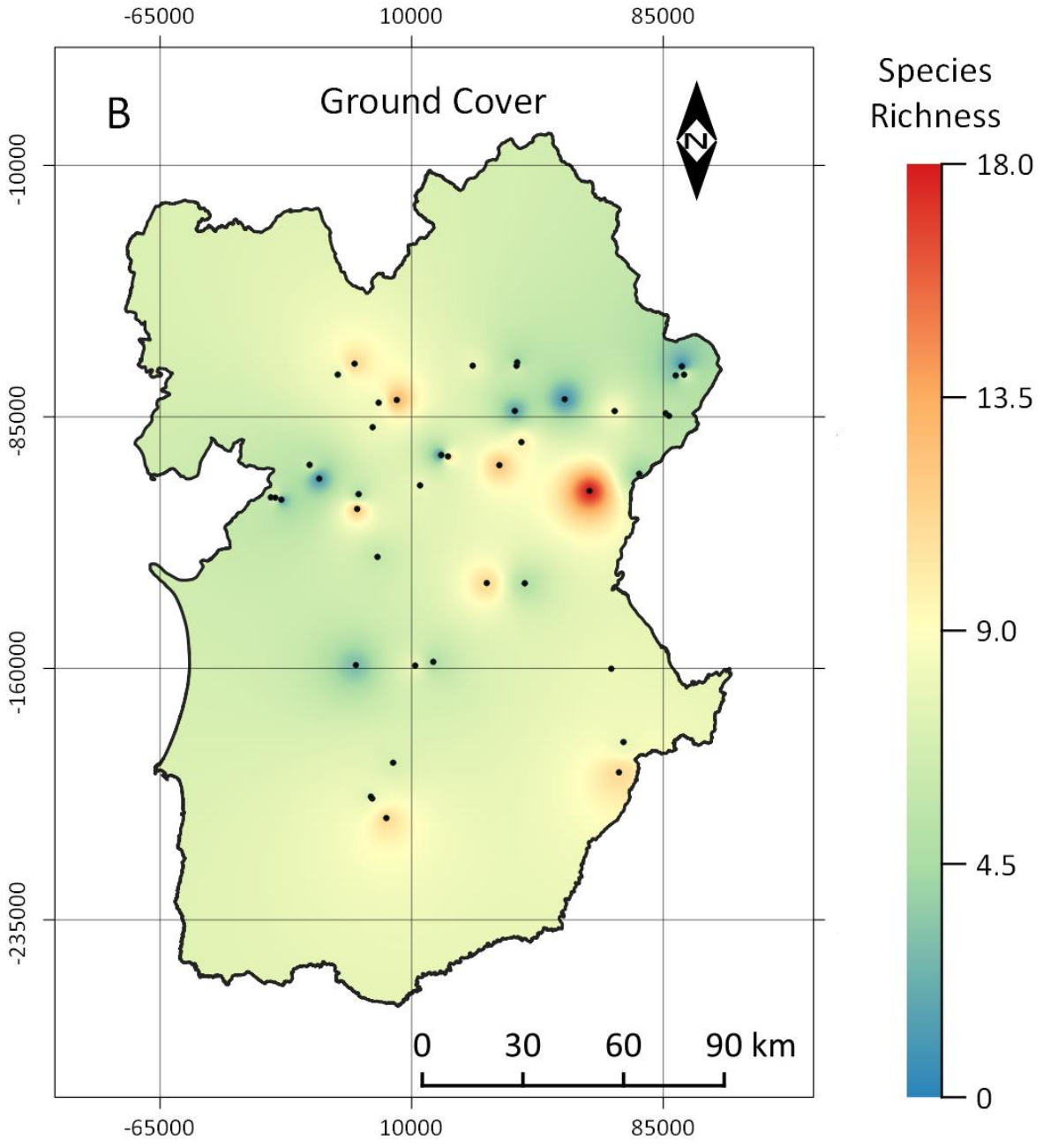

Figure 18: Map of global species richness identified in the ground cover sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). The samplings have closer values to the higher end of the species richness scale, being the mean 8.00. The sample point with the highest species richness was Q9 P134 ESP (Annex A – Table A5) – 18.00.

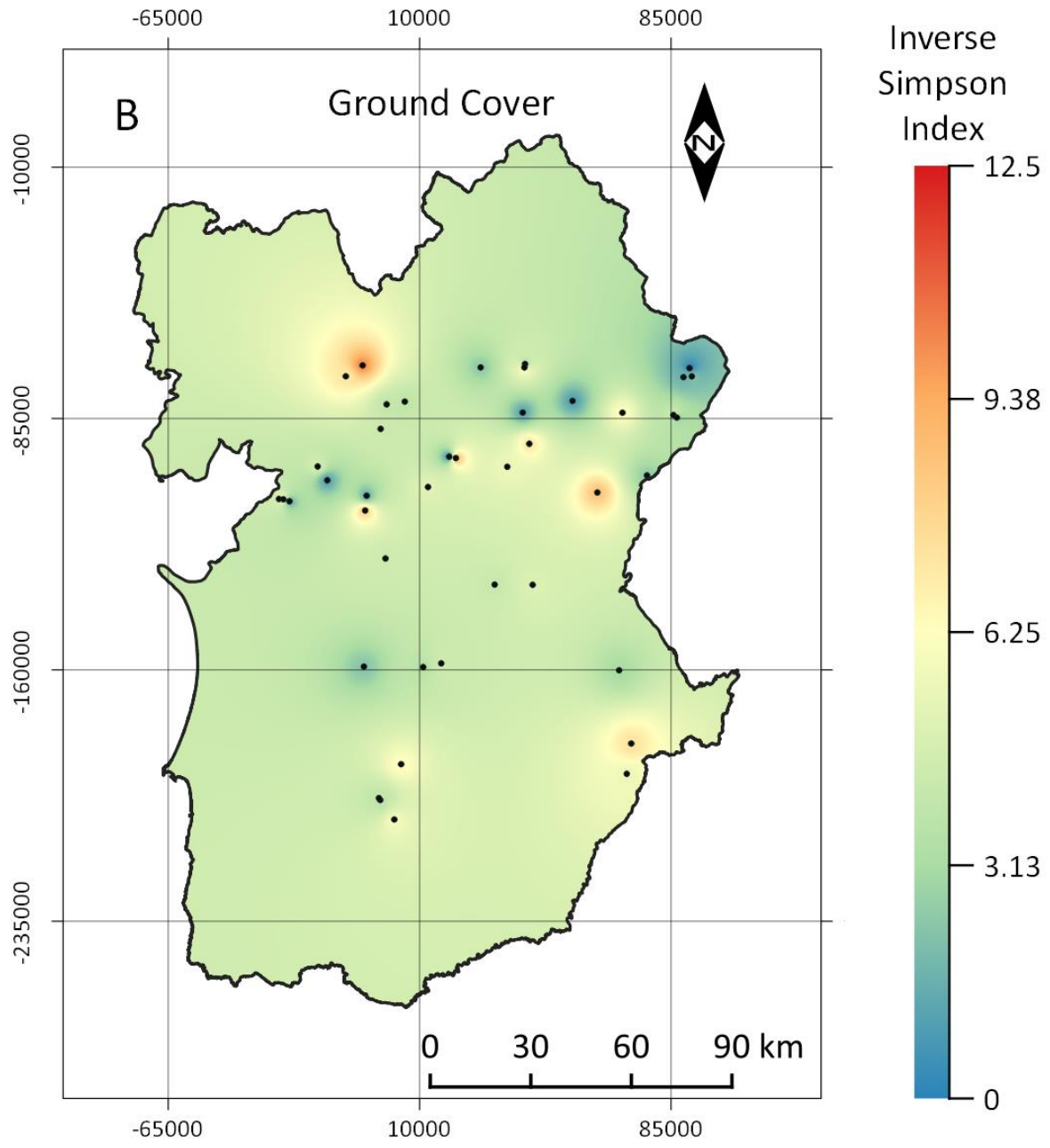

Figure 19: Map of global diversity index – inverse Simpson index - identified in the ground cover sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). The mean value of the sample spots for the Inverse Simpson Index was 4.40. The sample point with the highest value for this index was Q1 P151 ESP (Annex A – Table A5) – 10.29.

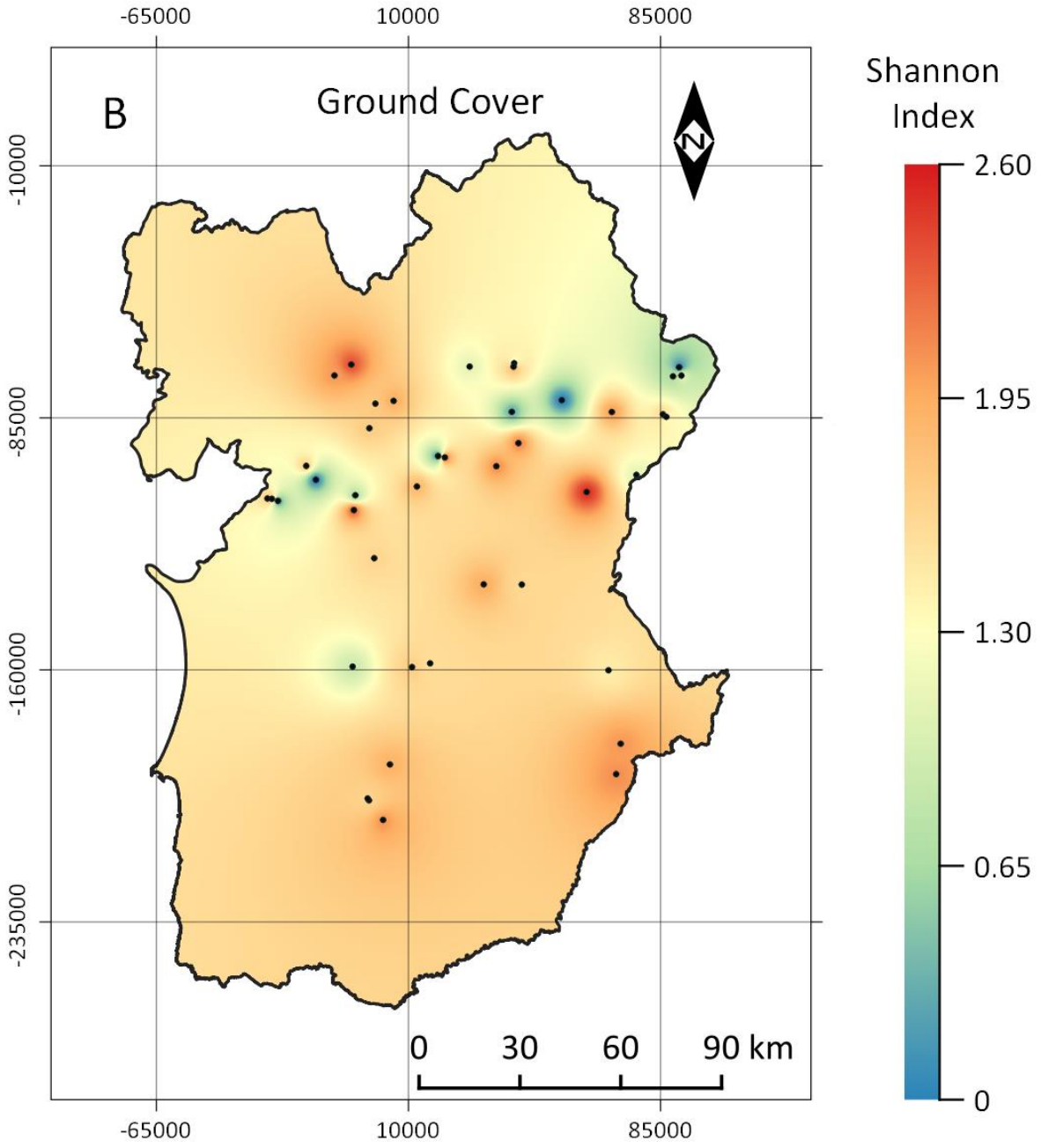

Figure 20: Map of global diversity index – Shannon index - identified in the ground cover sampling spots. The map is an extrapolation of the overall Alentejo region (not considering landscape use, and as such requires caution in interpretation beyond the exact location of the sampling spots). Most sample points scored in the high end of the Shannon Index scale being the mean 1.48. The sample spot with the highest value was Q9 P134 ESP (Annex A – Table A5) – 2.50.

3.4. Key predator species

From the 177 predator morphospecies identified in the samples, 58 of them share the olive canopy and ground cover ecosystems (Table 4). From those, 43 are from the Aranea Order, 2 Coleoptera and 13 Hymenoptera.

Table 4: List of predator species found in both sample sites – olive canopy and ground cover, with their respective number of individuals captured (N), dominance and frequency.

ORDER	FAMILY	PUTATIVE SPECIES	NO	NGC	DOMINANCE (%)	FREQUENCY (%)
Aranea	Araneidae	*Araneus* sp.	8	2	0.58	3.85
		Cyclosa sp.	1	1	0.12	1.28
		Hypsosinga albovittata	1	1	0.12	1.28
		Mangora acalypha	2	8	0.58	3.21
		Neoscona sp.	1	1	0.12	1.28
		Zygiella sp.1	9	9	1.04	10.26
	Clubionidae	*Clubiona* sp.1	9	13	1.27	10.90
	Gnaphosidae	*Berlandina* sp.	1	1	0.12	1.28
		Drassodes sp.	10	1	0.63	7.05
		Parasyrisca sp.	1	1	0.12	1.28
	Linyphiidae	*Drapetisca socialis*	1	1	0.12	1.28
		Frontinella sp.	1	1	0.12	1.28
		Leptyphantes sp.	1	2	0.17	1.92
		Linyphia sp.	4	1	0.29	3.21
		Neriene sp.	16	4	1.15	7.05
		Poeciloneta sp.	6	12	1.04	5.77
	Miturgidae	*Cheiracanthium* sp.	3	4	0.40	3.85
	Oxyopidae	*Oxyopes lineatus*	1	1	0.12	1.28
		Oxyopes sp.1	3	20	1.33	12.18
	Philodromidae	*Philodromus* sp.1	72	25	5.59	37.82
		Thanatus sp.	4	1	0.29	2.56
	Salticidae	*Chalcoscirtus* sp.	3	1	0.23	1.92
		Euophrys sp.	2	1	0.17	1.92
		Heliophanus sp.1	6	11	0.98	9.62
		Neon sp.	10	2	0.69	5.13
	Tetragnathidae	*Meta* sp.	1	5	0.35	1.92
		Metellina sp.	3	1	0.23	1.28
		Tetragnatha sp.1	49	2	2.94	7.05

ORDER	FAMILY	PUTATIVE SPECIES	NO	NGC	DOMINANCE (%)	FREQUENCY (%)
	Theridiidae	*Anelosimus* sp.	1	17	1.04	3.85
		Crustalina sp.	2	6	0.46	3.21
		Dipoena sp.	5	1	0.35	2.56
		Enoplognatha sp.	6	6	0.69	5.77
		Robertus sp.	1	1	0.12	1.28
		Rugathodes sp.	2	1	0.17	1.28
		Theridion sp.1	11	5	0.92	10.26
		Theridion sp.2	2	2	0.23	2.56
	Theridiosomatidae	*Theridiosoma* sp.	7	2	0.52	5.13
	Thomisidae	*Monaeses* sp.	3	1	0.23	2.56
		Ozyptila sp.	1	2	0.17	1.92
		Runcinia sp.	9	10	1.10	9.62
		Thomisus sp.1	6	28	1.96	12.82
		Tmarus sp.	6	2	0.46	5.13
		Xysticus sp.	5	8	0.75	6.41
Coleoptera	Coccinelidae	*Scymnus abietis*	2	2	0.23	2.56
		Scymnus mediterraneus	19	6	1.44	8.33
Hymenoptera	Formicidae	*Camponotus aethiops*	1	1	0.12	1.28
		Camponotus cruentatus	1	4	0.29	1.28
		Camponotus lateralis	43	2	2.60	19.87
		Crematogaster auberti	17	3	1.15	4.49
		Crematogaster scutellaris	129	26	8.94	25.00
		Crematogaster sordidula	5	46	2.94	10.26
		Formica subrufa	1	5	0.35	2.56
		Lasius brunneus	7	15	1.27	3.21
		Plagiolepis pygmaea	71	235	17.65	32.05
		Plagiolepis schmitzi	16	48	3.69	8.97
		Plagiolepis sp.	1	6	0.40	1.92
		Tapinoma sp.1 (*nigerrimum-simrothi* complex)	7	69	4.38	5.77

ORDER	FAMILY	PUTATIVE SPECIES	NO	NGC	DOMINANCE (%)	FREQUENCY (%)
Neuroptera	Chrysopidae	*Cunctochrysa* sp.	3	2	0.29	2.56

3.5. Molecular identification

Correct identification of a predator is a first essential step to access its potential impact on constraining a putative pest species population. Because Formicidae and Araneae were selected as the most promising predator groups, considering their abundance and diversity, only specimens from these groups were used in this step. Every morphotype with 5 individuals or more was used for their molecular identification through sequencing the established DNA barcode for insects -a short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene (usually a ca. 650 bp string corresponding to nucleotide positions 1490-2198 from the 5'- end of the COI of *Drosophila yakuba* (Burla, 1954) mitochondrial genome as a reference).

With the established criteria from the Formicidae and Araneae group, we separated 10 morphotypes and 29 respectively (Figure 21 and Figure 22).

Due to a laboratory contamination that was not possible to source during the experiment period and associated with time constraints, it was not possible to proceed with molecular identification of some of the chosen specimens and subsequent barcoding of their DNA (of the 29 specimens of the Araneae, only 4 were in good conditions and of 10 Formicidae, 8 of them were good to use). The results that were possible to obtain are represented in Annex A – Table A3 and Table A4. The sequences will be posted in the NCBI database for other studies *posteriori* to this dissertation. Efforts for continuing the DNA barcoding of the remain specimens will also made *posteriori* to this dissertation.

Considering the three sequences of Araneae obtained, one was confirmed as being correctly identified, and the other two showed overall low homologies and/or query cover (Annex A – Table A3). The morphospecies identified as *Meta* sp. is likely a misidentification and should be referred to as an Araneidae morphotype 1, the *Tetragnatha* sp.1 morphospecies remains in doubt (albeit highest homology with orb-weaver spider of the genus *Larinia* (voucher species, 92% homology for a query cover of 95%). Indeed, restricting the search to Tetragnathids, the best hit is also of a voucher species of *Tetragnatha* sp. (KF195571.1, 85% homology for a query cover of 97%).

As to the Formicidae, two morphotypes might have been misplaced at the genus level (Annex A – Table A3). In what refers to the morphospecies identified as *Crematogaster sordidula*, the BLAST search retrieved the sequence as belonging to *Pheidole pallidula* (99% homology for a query cover of 98%), within the same sub-family Myrmicinae. When searching NCBI for COI sequences of *Crematogaster sordidula*, no sequence could be found, raising the question on whether this specimen was morphologically misidentified or not. The same can be applied to *Plagiolepis schmitzi*, the BLAST search retrieved the sequence for *Plagiolepis manczshurica* (89.10% homology for a query cover of 95%), belonging to the same genus, however searching the NCBI, there were no sequences of *Plagiolepis schmitzi*. As to the specimen identified morphological as *Lasius brunneus*, it seems to be indeed a misidentification as both sequences are available at NCBI and the BLAST search retrieved *Linepithema humile* as the best hit (with 99% homology for a query cover of 98% against an 80% homology for a query cover of 94% with *Lasius brunneus* [LT977443.1]).


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Figure 21: Formicidae species used in the DNA barcoding. The identification code corresponds with the code given during the DNA extraction. (F1) *Plagiolepis pygmaea*. (F2) *Plagiolepis schmitzi*. (F3) *Formica subrufa*. (F4) *Crematogaster scutellaris*. (F5) *Crematogaster auberti*. (F6) *Crematogaster sordidula*. (F7) *Tapinoma* sp.1 (*nigerrimum-simrothi* complex). (F8) *Camponotus cruentatus*. (F9) *Camponotus lateralis*. (F10) *Lasius brunneus*.


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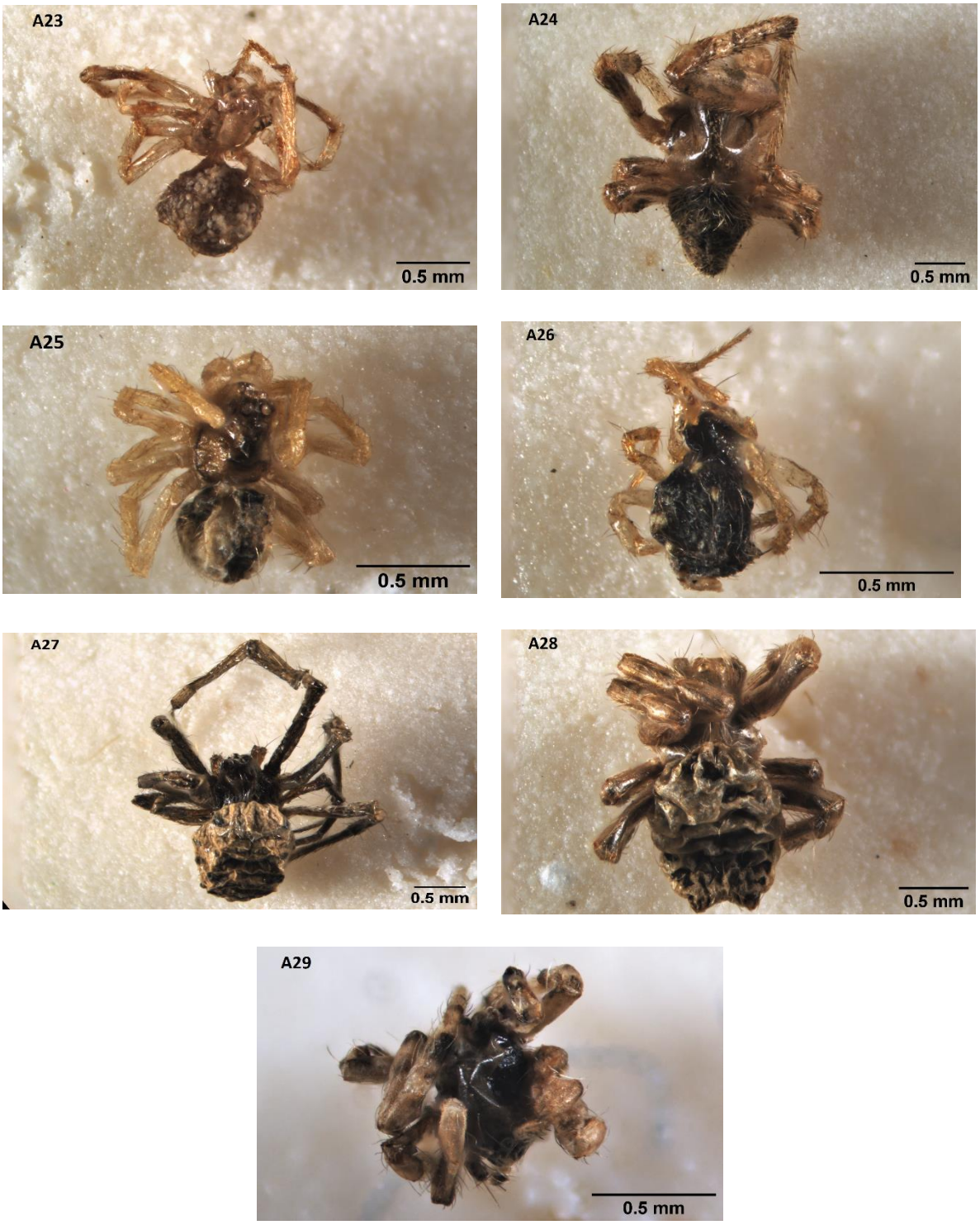

Figure 22: Araneae species used in the DNA barcoding. The identification code corresponds with the code given during the DNA extraction. (A1) *Drassodes* sp.(A2) *Oxyopes* sp. (A3) *Tmarus* sp. (A4) *Thomisus* sp. (A5) *Runcinia* sp. (A6) *Xysticus* sp. (A7) *Nigma* sp. (A8) *Clubiona* sp. (A9) *Tetragnatha* sp. (A10) *Meta* sp. (A11) *Trochosa* sp. (A12) *Cheiracanthium* sp. (A13) *Philodromus* sp.1. (A14) *Philodromus* sp.2. (A15) *Araneus* sp. (A16) *Zygiella* sp. (A17) *Zilla diodia*. (A18) *Mangora acalypha*. (A19) *Cyrtophora* sp. (A20) *Poeciloneta* sp. (A21) *Neriene* sp. (A22) *Frontinellina frutetorum*. (A23) *Dipoena* sp. (A24) *Theridion* sp. (A25) *Anelosimus* sp. (A26) *Crustalina* sp. (A27) *Enoplognatha* sp. (A28) *Episinus* sp. (A29) *Theridiosoma* sp.

3.6. Proof of principle

The 3 most abundant morphotypes of the Araneae and Formicidae were chosen. From this morphotypes 3 sets of pools of DNA of 5 specimens each were made.

The detection of *B. oleae* in the gut of the insects was positive in the Formicidae group selected and negative in the Araneae group used. The fragment obtained was sequenced and proven to be *Bactrocera oleae*. The *Tapinoma* sp.1 group (FPD13) appears to have some *B. oleae* mDNA in the Mix 3. The *Plagiolepis pygmaea* pool (FPD22) was also positive to the presence of mDNA of *B. oleae* in their digestive tract on all the mixes (Mix 1, Mix 2, Mix 3). The *Crematogaster scutellaris* pools (FPD31, FPD32, FPD33) had very strong positive results with Mix 3 for *B. oleae* mDNA and fainter results with Mix 2 on the FPD31 and FPD32 pools (Figure 23).

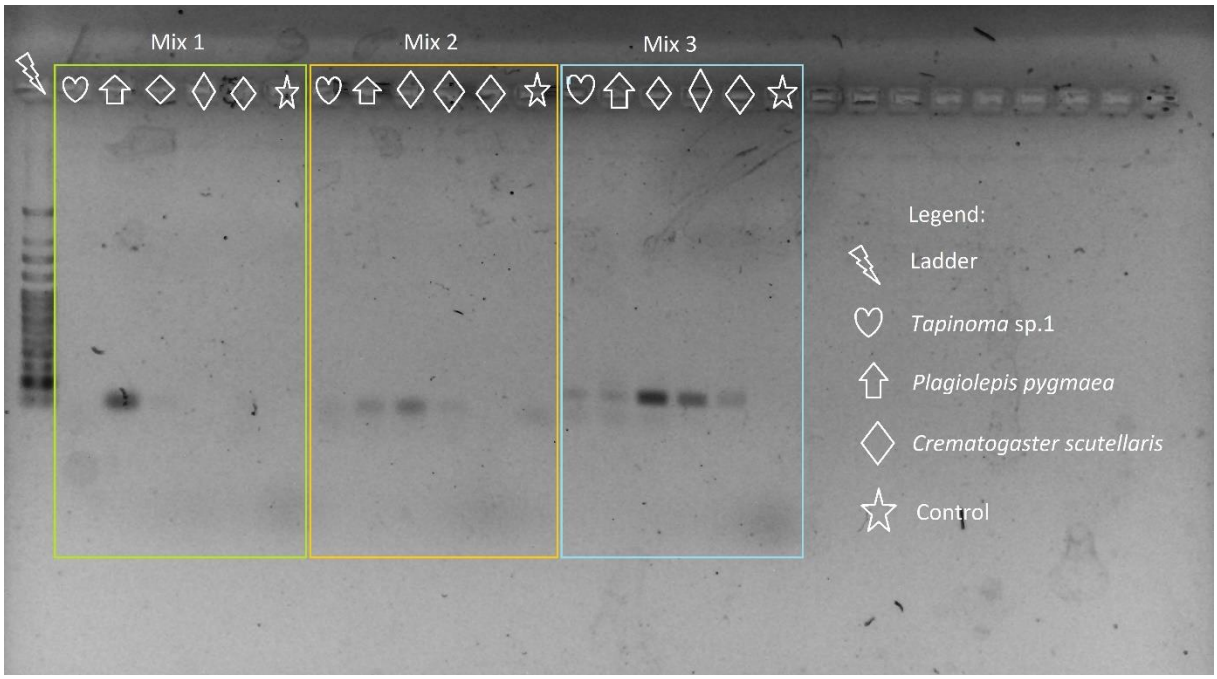

Figure 23: Agarose gel with the amplification of the COI of *B. oleae*. Each colour division is a different mix: green is Mix 1, yellow is Mix 2 and blue is Mix 3. On the far left is the ladder (with the bolt symbol). Each symbol represents a DNA pool. The heart is *Tapinoma* sp.1 FPD13 pool, the arrow is *Plagiolepis pygmaea* FPD22 pool and rhombus is the 3 pools of *Crematogaster scutellaris* (FPD31, FPD32, FPD33). The star is the negative control.

4. Discussion

4.1. Morphological identification and general considerations

The specimens used in this dissertation were all caught in the Autumn of 2016, between October and November. The summer of that year was characterized by very low precipitation and very high temperatures, which produced a very dry hot summer weather (IPMA, 2016). These conditions - very dry hot weather, affect negatively the overall diversity, abundance and richness of the morphospecies identified, on multiple levels, like reproduction, fitness, voltinism, etc (Ma, 2020). The way how the sample method was constructed did not allow a robust analysis between the sample spots and weather variables, since the available data were only the IPMA stations in the area (the temperature was not recorded in situ) and the flora species (ground cover) and sites (landscape) were also not characterized, all important variables to establish a relation between specific diversity and nature in situ.

The Autumn season (and also the Spring) is an ideal season to assess which predator and generalist's species exist in the region, that may control the olive fruit fly. The Autumn in particular, has a major effect in the predation of the pupae of *Bactrocera oleae* and posterior management and control of the next generation of individuals in the new crop year.

The main groups found in this dissertation were Araneae, Coleoptera, Heteroptera, Hymenoptera, Mantodea, Neuroptera, Opiliones and Pseudoscorpiones. These groups are also referred in previous studies of arthropods associated with Alentejo's olive culture and B. oleae. However, when lower taxonomic levels are considered such as families, this congruence was no longer observed. Some major families with many possible predators of the olive fruit fly, were not found, like Carabidae, Staphylinidae, Tenebrionidae, Forficulidae, Scolopendromorpha, (Rei, 2006; Dinis, 2015; Jimenez-Garcia, 2019). In the specific case of the Araneae order, Dinis (2015) described some families which were not found in the sampling, like Agelenidae, Dysderidae and Eresidae (Dinis, 2015). It is to be added that the method of capture of the arthropods needs to be considered when making comparative analyses. The vacuum of the canopies and the ground covers, as it was done, likely samples different specimens than, for instance, a pitfall trap or sticky traps. The sampling method selected thus induces an inherent bias making the studies difficult to compare directly.

4.2. Olive canopy samples

In the olive canopy samples 127 morphospecies were found from a total of 177 identified, being 69 of them found exclusively in these samples, nevertheless this does not mean that this morphospecies are not present in the ground cover samples, simply they were not capture in the sampling. The group with more morphospecies found in the olive culture was Araneae, which is different to the information of a previous work (Rei, 2006). The morphospecies with higher frequency in the olive canopy was *Philodromus* sp. 1 (38.94%). In Rei (2006), it was also one of the species with the highest frequencies. It is expected that the group with more species and the species with higher frequency, being from the Araneae order in the olive canopy, since the general habits of predation in this group deals with production of webs (adequate to capture flying insects, and so capturing adult olive fruit fly) (Jones, 1990).

The morphospecies with the highest total abundance was *Crematogaster scutellaris* with 129 individuals. This species was also found with high abundance in the work of Gonçalves et al. (2013) also in the Alentejo region in olive orchards. This abundance can be explained with their biology, since they belong to a group of ant species that do their nest in trees and forage in the canopy (Redolfi, 1999). The morphospecies with highest frequencies and total abundances were similar for the canopy samples (*Philodromus* sp.1, *Nigma* sp.1, *Chrysoperla carnea*, *Plagiolepis pygmaea*, *Crematogaster scutellaris*, *Camponotus auberti*), except for a few species. Overall, the frequency and the total abundance were

very low, with 48.8% of the morphospecies found on the canopy, having the lowest frequency and 41.7% of them having the lowest total abundance. The way the sampling was constructed, does not allow to infere on why the abundance values are so low for the majority of the species. Though it is possible to extrapolate that many species start to hibernate during the Autumn, leading to less prey available, and that leads to smaller populations of predators. Whether this is due to the season, or another variable, or if it is actually a constant finding all year around still needs to be researched.
In relation to the dominance in the canopy, *Chrysoperla carnea* was the more dominant morphospecies with 82.86%. As these sample sites had spontaneous plants around, and it was a hot autumn, it was a good breeding and feeding ground to stay in, since with hot weather the adults continue to lay eggs, also having a place to lay them – the leaves of the olive trees, and can feed of the pollen of the olive trees and plants nearby. Even not having as much readily available pollen produced by the autumn plants, they can still feed on the pollen settled in the environment (Villa, 2019). The morphospecies with the lowest dominance were all from the Araneae order (30.7% of all the morphospecies identified in the canopy). Most of these, were morphospecies that had only 1 individual present in all the olive canopy samples.
For the values of diversity calculated for the olive canopy samples, all of them except the Shannon index, had the same 2 points as its lowest value. These can be explained with the fact that in those 2 points, there were not predators present. In the case of the Shannon index there were 16 sample points with the lowest value, in this case all of them had or the richness + abundance equal to 0 or richness and abundance equal to 1. The highest inverse Simpson index value and the highest Shannon index value belong to the same sample spot (Q1 P150 OLI), which reveal that it is the spot with the best diversity-evenness relation. But it is not possible to correlate with environmental and climatic variables, so it's not possible to access the underlying causes of this values. The same can be said for the highest values for the richness and global abundance (both of them had different points with the higher value). Every parameter calculated of diversity, showed that the generality of the samples had low diversity, with 11.5% with the lowest global abundance, 15.9% the lowest richness, 12.4% the lowest inverse Simpson index and 14.2% with the lowest Shannon index.

4.3. Ground cover samples

In the ground cover samples 107 morphospecies were found from the total of 177 identified, being 49 of them found exclusively in these samples, nevertheless this does not mean that this morphospecies are not present in the olive canopy samples, simply they were not capture in the sampling. Like in the samples of the canopy, the Araneae order is the one with more species present in the environment. In other studies, done with predator arthropods of the soil, this group was also one of the prevalent (Rei, 2006; Gkisakis, 2014; Dinis, 2015; Gonçalves, 2017; Jimenez-Garcia, 2019). Opposing to what was observed in the canopy, and as expected, spider's species comprised mainly predators with a hunting behaviour, such as Lycosidae, Salticidae and Thomisidae., which is in line with an environment of low height plants and bare soil, where the web prevalent families have a disadvantage, even though, many species of those families still appear. The morphospecies with the highest frequency and total abundance was *Plagiolepis pygmaea*, with a frequency of 55.81% and a total abundance of 230 individuals. In the studies referred before, the ants more abundant or with the highest frequency in the soil were *Pheidole pallidula* (Gonçalves, 2013), *Tapinoma nigerrimum* (Dinis, 2015) and *Crematogaster scutellaris* (Rei, 2006), all these morphospecies or genera appeared on the samples with high frequencies and abundance. The more dominant morphospecies in the ground cover samples were *Ameles spallanzania*, *Cunctochrysa* sp. and Opiliones Morphotype sp.1, with 100.0%. It has to be said these morphospecies had the highest dominance, because they were the single representatives of their order.
For the values of diversity calculated for the ground cover samples, all of them except the Shannon index, had also the same 2 points as its lowest value. These can be explained with the fact that in those

2 points, there were not predators present. In the case of the Shannon index there were 5 sample points with the lowest value, in this case all of them also had or the richness + abundance equal to 0 or richness and abundance equal to 1. The highest inverse Simpson index value and the highest Shannon index value belong to different sample points, which is different from what happened in the olive canopy samples. However, the sample point with the highest richness is the same with the highest Shannon index, which could mean that besides having the highest richness of species of the ground cover samples, also has a high level of evenness between the species in that sample. This information was not possible to correlate with environmental and climatic variables, so it's not possible to assess the underlying causes of these values. The same can be said for the highest values of the inverse Simpson index and the global abundance. Every parameter calculated of diversity, showed that the generality of the ground cover samples just like the olive canopy samples, had low diversity, with 11.6% with the lowest global abundance, 23.3% the lowest richness, 4.7% the lowest inverse Simpson index and 11.6% with the lowest Shannon index.

4.4. Key predator species

The morphospecies considered as key; were the ones that were found in common between the two spots – olive canopy and ground cover, and so considered the more probable ones to go between the two landscape *strata* and predate the fly. Even though that was decided as the major reasoning to consider them key to the control, it is to be referred that is unlikely that the Coccinellid added to this group actually predate the olive fruit fly. They were in general very small in size and in the case of this group, they tend to predate smaller prey in size, comparing to them (Evans, 2009). If size would not be a constraint, however, they could predate later instar of the olive fruit fly, when they decide to descend to the soil for the formation of the pupae.

Even though, in the table made with prospect key predator species, only appear *Cunctochrysa* sp. as a key predator of the Neuroptera order, it is logical to include all the Neuroptera identified in the samples (*Chrysoperla carnea*, *Cunctochrysa baetica* and *Chrysoperla* sp.), since this morphospecies that appeared in both spots was in larvae stage, so it is possible that the same might happen with the other morphospecies of the order.

Beside these two group the main key predator species belong in the Araneae and Formicidae groups. These are the groups more promising for management of the fruit fly. Given their methods of hunting and behaviour, they probably are suitable for different life stages of the olive fruit fly, the ants predating mainly the pupae and the spiders the adults.

So, it is important to think of strategies to promote these groups and use them to aid the control of *B. oleae*. Factors linked to the type of management in farming, climate and landscape affect the variability of arthropods (Gkisakis, 2018), and so influence the predators in the culture. It is then important to assess the agroecological nature of a local, to better manage it. For instance, orchards with biological management might have an edge, since it was observed that this type of management, increases the predation rates of pupae of *B. oleae* (Picchi, 2017). A study of Ortega et al. (2018), also refers the importance of the landscape in this case more specifically for the predation of *B. oleae*. It also adds that the soil is very important for the predation of the olive fruit fly pupae, and advices the reduction of the soil management particularly during the Autumn (the season of highest pupae predation) and the preservation of scrublands surrounding the olive orchards (Ortega, 2018).

The mix of fruit trees and vegetable plots can also influence the presence of predators, by increasing the levels of pests, but not the predators. Diversity of plants is important for the overall diversity of arthropod species, but the right kind of diversity, since some plants might enhance pest population sizes and not the auxiliary arthropods populations, which is something to have in consideration for the promotion of natural enemies' arthropods (Imbert, 2020). However, in the case of ground cover and flowering plants adjacent to the plots and locals of orchards, their presence and the type of plant might

enhance the abundance and diversity of auxiliar and predator arthropod species, and help in the control of the olive fruit fly (Amoabeng, 2018; Carpio, 2019; Karamaouna, 2019; Patt 2020).
The use of chemical agents affects the predators besides the target pest. Studies done on the subject have shown a decrease of predators using kaolin (González-Núñez, 2008) and even the products used in organic farming, like copper oxychloride, azadirachtin and rotenone, have negative effects on the arthropods present in the olive orchards (Iannotta, 2007).
In the case particularly of the Araneae there are additional measures that can be used and considered to promote their diversity, abundance and presence in the olive orchards to control *B. oleae* (in its adult phase). The presence of stones in the soil of olive groves promote the presence of spiders, giving them hiding spots during hibernation and aestivation which is crucial for the maintenance of the populations (Benhadi-Marin, 2018). It is to highlight once more, the importance of the landscape, on the abundance and richness of spider species. The presence of Mediterranean garrigue in the surroundings of the farming plots affects the composition of the spider's communities. On, the other hand, the increase of woods near the orchards, enhances the abundance of flies (Picchi, 2016). Another way to maintain and increase the abundance of Araneae is having non-prey food available. If immature spiders have access to honeydew, pollen and nectar, the probability of surviving, increases (Benhadi-Marin, 2019).

4.5. Molecular identification

The molecular identification was only partially completed due to laboratory contamination and time constraints. The specimens in which the molecular identification did not corroborate the morphological one, is a result of the author's inexperience with arthropod identification but also with lack of information in the databases. As a rule of thumb, the online database used allowed species identification when our sequence matched the available reference sequence with an identity value greater than 97%, given that intraspecific genetic distance should not exceed 3% (Hebert, 2003).
The specimen identified as *Clubiona* sp., had its highest match with a sequence identified as Araneidae sp. but it must be referred that the third highest hit identified the sequence as *Clubiona genevensis* (that is the species name given in NCBI, but the accepted name is *Porrhoclubiona genevensis* (L.Koch, 1866)) suggesting a correct identification of the specimen genera. To assess if it is this species, in the future the DNA barcoding should be repeated with another individual of the sample identified as *Clubiona* sp.
As referred in the results, the morphospecies identified morphologically as Meta sp. is a case of actual misidentification and the individual belongs to the Araneidae family instead of the Tetragnathidae. Many individuals of the Araneae order were not in the best of conditions, in some of the cases missing parts which were fundamental for the morphological identification. Furthermore, some characters for the morphological identification are slightly subjective (like shading, relative sizing, etc), particularly when dealing with not freshly sampled specimens. For a better identification the pedipalps of the morphotypes could be extracted, since they are one of the better parts to identify families, genus and species, but there were time and equipment limitations, and often missing pedipalps in some of the specimens. Regarding the other Araneae morphospecies, doubt remains if *Tetragnatha* sp.1 was a correct identification since both possibilities (being from the genus *Tetragnatha* or *Larinia*, both from different families) have lower homologies than 97% (*Tetragnatha* 85% and *Larinia* 92% of homology respectively), and thus, we have to rely so far on the morphological identification and try to extend the effort to the analysis of the genitalia of the specimens.
In the case of the Formicidae, two of the specimens used to identify molecularly (*Crematogaster auberti* and *Crematogaster scutellaris*), gave as their highest hit the same sequence of *C. auberti* in the NCBI. There are two possible explanations: the author identified incorrectly, and produce a division of the *Crematogaster auberti* species in two, or the other possibility it was a misplaced single individual of the *C. auberti* species in the container dedicated to *Crematogaster scutellaris*. Since *C. scutellaris* is a very common and abundant species in Alentejo region (FCTVIVA, 2020), it would have been curious not

having that species present in the samples, so it is more probable that it was a misplaced individual in the wrong container. It can be solved, repeating the process of DNA barcoding with other individuals identified as *C. scutellaris* in the sample.

The specimen identified as *Plagiolepis schmitzi* had as its highest hit *Plagiolepis manczshurica* Ruzsky, 1905, which is a subspecies of *Plagiolepis pygmaea* distributed usually in China, Russia and the palaearctic region (AntWeb, 2016). It was not possible to find information about its presence in Portugal or the Mediterranean basin, but one could suggest that is the establishment of an exotic species in the region, since this species had many individuals collected in many sample spots. However, the most likely explanation refers to lack of information on the databases: no sequence of the species *Plagiolepis schmitzi* is available in the database of the NCBI and the search does retrieve the sequence available with higher homology. *P. schmitzi* distribution is given to all Iberian Peninsula, and is widely distributed in Portugal.

The other Formicidae species, *Tapinoma simrothi* Krausse, 1911 was correctly identified since the morphological identification put it inside a complex of 2 species – *Tapinoma nigerrimum* Nylander, 1856 and *Tapinoma simrothi*, helping in this case separate the complex in an actual single species. For the morphospecies identified as *Crematogaster sordidula*, the highest hit in the BLAST was *Pheidole pallidula* (Nylander, 1849). In this case, the problem is similar to the *Plagiolepis schmitzi*, because the species does not correspond but belongs to the same subfamily – Myrmicinae. Since in this case there are also no DNA sequences for *Crematogaster sordidula*, it is difficult to assess if it is a misidentification or the absence of the species sequence in the NCBI. To eliminate this problem the individuals for this morphospecies should be taken to a specialist and compared with more than one collection of reference, in the case of confirming as *C. sordidula*, the sequence should be added to the NCBI belonging to the species for the use of the research community. The morphospecies identified as *Lasius brunneus*, its higher hit was *Linepithema humile*, which seems that it was actually a misidentification since both sequences *(Lasius brunneus* and *Linepithema humile*) are present in NCBI. This species – *Linepithema humile*, is an exotic species from South America widely dispersed in Portugal (Collingwood, 1979)

4.6. Proof of principle

With the selected Formicidae it was possible to identify *Bactrocera oleae* mtDNA in their gut, proving that predator and generalists' ants, predate on the olive fruit fly during the Autumn season. Might be a group to have in consideration for management and control of this pest.

The Araneae selected did not show the presence of *B. oleae* mtDNA, but that does not indicate *per se* that those individuals actually did not eat any form (pupae or adult) of the olive fruit fly. The DNA in the individual's digestive system is subject to degradation and with the current methods it can only be detected until a certain time after consumption. In the study of Rejili, et al (2016) it could be detected in the gut, *Bactrocera oleae* DNA until 16h after feeding. A similar digestion period was used in the work of Panni et al (2018), in this case detecting olive fruit fly DNA until 18h after feeding. In the study of Lantero et al (2017) the period of detection of *B. oleae* DNA in the gut was larger, going until the 72h after feeding (in this case made possible with the aid of BSA). So, it is possible that the Araneae individuals eat the olive fruit fly, but a longer time before being sampled. Other variable that might influence the time of digestion until detection is the group on itself, since all these studies were done in carabid species and in a specimen of Staphylinidae (Albertini, 2018). The Araneae as a different group might require a different approach, both in time and eventually in protocol as there might also be available other metabolites that can potentially inhibit PCR amplification of the probed olive fruit fly mtDNA. It can be something to consider in the future and as so this group should not be discarded as tool to olive fruit fly management and control.

4.7. Final considerations and future perspectives

The Mediterranean region is the largest area with suitable conditions for the growth of the olive tree and its farming. Having such a niche climate and ecosystem, only present in the Mediterranean basin and a part of California, is especially important to prospect how the future conditions might alter it, and how does changes would impact on the growing and farming of olive trees. It has to be considered how climate change will alter the environment and affect the olive tree, because alterations in the physiology, biology and life cycle of the tree will influence how its pests interact. Likewise, it will impact on the extant arthropods that rely on the predation of a specific pest population. The current studies regarding how climate change will act upon the olive orchards refer to an increase of the crop, of about 25% for northern parts and higher altitude of the producer countries (Gutierrez, 2009; Tanasijevic, 2014); the flowering season is expected to be anticipated and crop evapotranspiration is expected to increase an 8%. Overall is expected that net irrigation of the crop will increase and the number of rainfed olive crops will decrease and be highly restrained (Tanasijevic, 2014), leading to a negative impact on the viability of this crop in the southern regions of the Iberia Peninsula (Fraga, 2019).

The olive fruit fly has a lower thermal tolerance compared with its host, so it is expected that southern and low altitude regions will have lower levels of infestation (Gutierrez, 2009). Nonetheless, this species is likely to move up north following the expansion of the area occupied by the olive tree. Besides distribution of the species, climate change can also influence population dynamics, interaction with its host, etc. These altered factors of influence can also be applied to its natural enemies, adding alterations to arthropod diversity and emergence of new biotypes of insect pests (Sharma, 2018). The warming of the soil also affects the arthropods of the soil, decreasing plant richness and increasing the dominance of certain groups or species over others, overall changing the composition of the community and its biomass (Robinson, 2017). Climate change can also either promote or decrease the severity of pest outbreaks and disrupt trophic interactions (Pureswaran, 2018). All these relations, interactions and factors have to be accounted for the current and future management of the olive tree crop, so they can alter the effectiveness of biological management programs. With this conditions in mind it is suggested by Nechols(2020) to conduct surveys for non-target species in areas currently undergoing climate change (like the Alentejo region, which belongs to the south of Iberia Peninsula), determine the range tolerance to temperature and precipitation of natural enemies, incorporate climate data into arthropods models and long term assessment and documentation of the impact of the climate change in the pests/natural enemies and efficacy of the management programs on them, ideally these program needed to be well funded involving interdisciplinary multitude of scientists.

In regard to the main aspects of this work, a varied group of natural predators of *B.oleae* was identified, both in the olive canopy where the adults are more present, and in the ground cover, where the pupae stage is prevalent at the time of sampling. Of all the groups identified, the ones with greater potential to control the olive fruit fly are Araneae and Formicidae, which were in general the most frequent, abundant and diverse. It was shown effective predation on the olive fruit fly from ants, thought the identification of *Bactrocera oleae* DNA in the digestive tract of some of the species of Formicidae. Even though it was not possible to have the same positive result with the Araneae species, predation can by no means be discarded due to the discusses inherent limitations of the method (e.g. detection limit, time since predation, etc).

To give immediate continuation to this work, and fill in the gaps, the DNA barcoding of the selected speciesis expected to be finished and all sequences deposited to the NCBI. New attempts will be made with the gut analysis method, focusing mainly on the spiders both using other collected individuals and optimizing the detection protocols. In the long run, it would also be interesting to perform time repetitions of the same framework using this and complementary sampling and taken care to collect meaningful environmental variables.

Promotion of the diversity and abundance of auxiliary arthropods' fauna should be an aim in olive pest management: it is important the type of management, the type of farming (since on each type of farming, the way it is managed varies greatly), the existence of natural plants and the species of plants near the crop, the cross farming of different crops, the use of chemicals and/or other additives (even the ones allowed in organic farming) and also the presence of rocks as hiding places for the Araneae order and the presence of non-prey food also for the Araneae.

5. Conclusion

In this study 177 predators and generalists were identified. The orders with more species were Araneae and Hymenoptera. They were also the groups that in general had higher diversity, abundance and frequency, and were selected to proceed with molecular identification (DNA barcoding) and gut analyses (proof of principle).

The samples were separated by host (olive canopy or ground cover), since they were considered different *strata* in which enemy arthropods would prey on different stages of the olive fruit fly. The morphospecies with the highest frequency in the olive canopy was *Philodromus* sp.1, the most abundant was *Crematogaster scutellaris* and the dominant was *Chrysoperla carnea*. In the case of the morphospecies of the ground cover samples, the most frequent and most abundant was *Plagiolepis pygmaea*, and the most dominant were *Cunctochrysa* sp., *Ameles spallanzania* and Opiliones Morphotype sp.1, but just because they were the only representative of the group. In terms of ecological index measures, most sampled points of both *strata*, had low abundance, richness and diversity. It was not possible to compare with climatic and biotic factors (the species of plants that composed the samples of ground cover) due to the way the sampling was structured.

Effective predation of the ant species *Tapinoma* sp.1, *Plagiolepis pygmaea* and *Crematogaster scutellaris* on the olive fruit fly was shown, indicating the ant's community potential in aiding lowering the numbers of this potential pest population. The same proof could not be delivered for the spiders tested, but their presence and predation mode suggest that they have the same potential. Further efforts are needed, as such a proof-of-principle could be the needed evidence to start managing the crop towards augmentation of predators' numbers and diversity.

Since several potential and effective natural enemy predators of the olive fruit fly are present in the olive crop, it is advisable to promote and increase their frequency and diversity. It is suggested for the *B. oleae* management, the decrease the use of pesticides, the promotion of natural plants near the crops, caution with the mix of crops done in a same plot (if that is the usual case in a determined production), increase of rocks in the soil and non-prey food for the Araneae, reduction of soil management mainly in the Autumn season, increasing the shrublands, etc.

In recent years with the increase of organophosphate resistance by the olive fruit fly, the imminent ban of its use in Europe and the importance given by the public to biological products, many studies considering alternative methods to *B. oleae* management have started to appear. Some studies on natural enemies have been done, but they are still by far not enough, and might this one adds to the subject and increase the available information and point future research direction for a better pest integrated management.

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Annex A

Table A1: List of predator species found exclusively in the samples of olive canopy, with their respective number of individuals captured (N), dominance and frequency (in the olive canopy samples).

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
Aranea	Anyphaenidae	*Anyphaena* sp.	4	0.30	1.77
	Araneidae	*Cyrtarachne ixoides*	1	0.07	0.88
		Cyrtophora sp.	11	0.82	4.42
		Larinia lineata	1	0.07	0.88
		Larininoides sp.	2	0.15	1.77
		Mangora sp.	1	0.07	0.88
		Singa sp.	2	0.15	1.77
		Zilla diodia	11	0.82	5.31
		Zygiella sp.2	1	0.07	0.88
	Clubionidae	*Clubiona* sp.2	2	0.15	0.88
	Dictynidae	*Lathys humilis*	1	0.07	0.88
		Mastigusa arietina	2	0.15	1.77
		Mastigusa sp.	2	0.15	1.77
		Nigma sp.1	43	3.22	17.70
		Nigma sp.2	1	0.07	0.88
	Gnaphosidae	*Callilepis* sp.	1	0.07	0.88
		Civizelotes sp.	1	0.07	0.88
		Gnaphosa sp.	1	0.07	0.88
		Haplodrassus sp.	2	0.15	1.77
		Leptodrassus sp.	5	0.37	4.42
		Scotophaeus sp.	1	0.07	0.88
		Setaphis sp.	1	0.07	0.88
	Linyphiidae	*Frontinellina frutetorum*	6	0.45	0.88
		Microlinyphia sp.	2	0.15	1.77
		Peponocranium sp.	1	0.07	0.88
	Lycosidae	*Pardosa* sp.	1	0.07	0.88
	Mimetidae	*Ero* sp.	1	0.07	0.88
	Philodromidae	*Philodromus emarginatus*	1	0.07	0.88
		Philodromus sp.2	21	1.57	15.04
		Philodromus sp.3	1	0.07	0.88
	Pisauridae	*Dolomedes fimbriatus*	2	0.15	1.77
	Salticidae	*Cyrba algerina*	2	0.15	1.77
		Cyrba sp.	1	0.07	0.88
		Dendryphantes sp.	1	0.07	0.88
		Evarcha sp.	2	0.15	1.77
		Hasarius adansoni	1	0.07	0.88
		Heliophanus sp.2	1	0.07	0.88
		Icius hamatus	1	0.07	0.88
		Leptorchestes sp.	3	0.22	2.65
		Marpissa sp.	1	0.07	0.88

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Pellenes sp.	1	0.07	0.88
		Pseudeuophrys vafra	1	0.07	0.88
		Saitis barbipes	2	0.15	1.77
		Salticus sp.	2	0.15	1.77
	Sparassidae	*Olios* sp.	4	0.30	3.54
	Tetragnathidae	*Pachygnatha* sp.	4	0.30	2.65
		Tetragnatha sp.2	2	0.15	0.88
	Theridiidae	*Episinus* sp.	6	0.45	2.65
		Neottiura bimaculata	1	0.07	0.88
	Thomisidae	*Diaea* sp.1	2	0.15	1.77
		Diaea sp.2	1	0.07	0.88
	Zodariidae	*Amphiledorus* sp.	1	0.07	0.88
Coleoptera	Coccinelidae	*Clitostethus arcuatus*	1	2.00	0.88
		Coccinella septempunctata	1	2.00	0.88
		Propylea quatuordecimpunctata	2	4.00	0.88
		Scymnus apetzi	2	4.00	0.88
		Stethorus punctillum	22	44.00	12.39
Heteroptera	Anthocoridae	*Acompocoris pygmaeus*	1	0.22	0.88
		Anthocoris nemorum	4	0.89	3.54
Hymenoptera	Formicidae	*Camponotus foreli*	1	0.25	0.88
		Camponotus pilicornis	1	0.25	0.88
		Camponotus ruber	1	0.25	0.88
		Camponotus sicheli	1	0.25	0.88
Neuroptera	Chrysopidae	*Chrysoperla carnea*	58	82.86	30.09
		Chrysoperla sp.	5	7.14	4.42
		Cunctochrysa baetica	4	5.71	2.65
Opiliones	Phalangiidae	*Dicranopalpus* sp.	1	33.33	0.88
		Odiellus sp.	1	33.33	0.88
	Sclerosomatidae	*Leiobunum* sp.	1	33.33	0.88

Table A2: List of predator species found exclusively in the samples of ground cover, with their respective number of individuals captured (N), dominance and frequency.

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
Aranea	Araneidae	*Gibbaranea* sp.	1	0.21	2.33
		Zilla sp.	1	0.21	2.33
	Gnaphosidae	*Zelotes* sp.	1	0.21	2.33
	Lycosidae	*Hygrolycosa rubrofasciata*	3	0.61	6.98
		Trabaea sp.	3	0.61	4.65
		Trochosa sp.	6	1.23	11.63

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
	Oxyopidae	*Oxyopes* sp.2	2	0.41	4.65
		Oxyopes sp.3	1	0.21	2.33
	Philodromidae	*Philodromus* sp.4	2	0.41	4.65
		Thanathus oblongiusculus	2	0.41	4.65
		Tibellus sp.	3	0.61	6.98
	Pisauridae	*Pisaura mirabilis*	1	0.21	2.33
	Salticidae	*Euophrys frontalis*	1	0.21	2.33
		Phlegra sp.	1	0.21	2.33
		Salticus cingulatus	1	0.21	2.33
	Selenopidae	*Selenops* sp.	2	0.41	4.65
	Theridiidae	*Euryopis* sp.	3	0.61	4.65
		Neottiura sp.	1	0.21	2.33
		Steatoda sp.	1	0.21	2.33
		Theridion sp.3	2	0.41	2.33
		Theridion sp.4	1	0.21	2.33
	Thomisidae	*Coriarachne* sp.	4	0.82	4.65
		Synema sp.	1	0.21	2.33
		Thomisus sp.2	1	0.21	2.33
	Uloboridae	*Uloborus* sp.	1	0.21	2.33
	Zodariidae	*Selamia* sp.	4	0.82	6.98
Coleoptera	Coccinelidae	*Hippodamia variegata*	1	11.11	2.33
Heteroptera	Miridae	*Compsidolon* sp.	1	0.24	2.33
		Deraeocoris serenus	1	0.24	2.33
		Dicyphus annulatus	1	0.24	2.33
		Dimorphocoris sp.	2	0.47	2.33
		Orthotylus sp.	32	7.51	23.26
	Nabidae	*Nabis* sp.	2	0.47	4.65
Hymenoptera	Formicidae	*Aphaenogaster senilis*	1	0.17	2.33
		Camponotus barbaricus	1	0.17	2.33
		Camponotus piceus	1	0.17	2.33
		Cardiocondyla sp.	4	0.67	2.33
		Lasius alienus	3	0.50	2.33
		Temnothorax sp.1 (*recedens* complex)	1	0.17	2.33

ORDER	FAMILY	PUTATIVE SPECIES	N	DOMINANCE (%)	FREQUENCY (%)
		Tetramorium caespitum	1	0.17	2.33
		Tetramorium semilaeve	1	0.17	2.33
		Tetramorium sp.1 (*simillimum* complex)	1	0.17	2.33
Mantodea	Mantidae	*Ameles spallanzania*	1	100.00	2.33
Opiliones	-	Opiliones sp.1	2	100.00	2.33
Pseudoscorpiones	Cheliferidae	*Hysterochelifer tuberculatus*	2	6.06	2.33
	Chernetidae	*Pselaphochernes* sp.	1	3.03	2.33
	Chthoniidae	*Chthonius* sp.	2	6.06	4.65
	Geogarypidae	*Geogarypus nigrimanus*	19	57.58	9.30
		Geogarypus sp.	9	27.27	11.63

Table A3: Selected predator specimens collected in sampling sites of olive trees and cover crops with GenBank accession numbers of COI. As a rule of thumb, the online database used allowed species identification when our sequence matched the available reference sequence with an identity value greater than 97%, given that intraspecific genetic distance should not exceed 3% (Hebert et al., 2003, https://doi.org/10.1098/rspb.2002.2218).

PUTATIVE SPECIES	MAX SCORE	QUERY COVER	E VALUE	PERCENT IDENTITY	ACCESSION	IDENTITY
Clubiona sp.1	1105	98%	0.0	98.11%	MK644552.1	Araneidae sp.*
Cheiracanthium sp.	845	97%	0.0	91.82%	KY269456.1	*Cheiracanthium erraticum* (Walckenaer, 1802)
Meta sp.	1122	97%	0.0	99.84%	MT607784.1	*Kochiura aulica* (C. L. Koch, 1838)
Tetragnatha sp.1	843	95%	0.0	91.87%	JN306125.1	*Larinia phthisica* (L. Koch, 1871)
Camponotus lateralis	1099	92%	0.0	100.00%	KX954289.1	*Camponotus lateralis*
Crematogaster auberti	1109	94%	0.0	99.19%	FJ824415.1	*Crematogaster auberti*
Crematogaster scutellaris	985	87%	0.0	98.57%	FJ824415.1	*Crematogaster auberti*
Crematogaster sordidula	1168	98%	0.0	99.38%	EF518381.1	*Pheidole pallidula*

PUTATIVE SPECIES	MAX SCORE	QUERY COVER	E VALUE	PERCENT IDENTITY	ACCESSION	IDENTITY
Iberoformica subrufa	1061	89%	0.0	100.00%	KX954286.1	*Iberoformica subrufa*
Lasius brunneus	1160	98%	0.0	99.84%	NC_045057.1	*Linepithema humile*
Plagiolepis schmitzi	730	95%	0.0	89.10%	JQ681047.1	*Plagiolepis manczshurica*
Tapinoma sp.1 (nigerrimum-simrothi complex)	1048	93%	0.0	99.82%	KY426465.1	*Tapinoma simrothi*

*The highest species hit was *Clubiona genevensis* with a 94% query cover and 93.30% percent identity.

Table A4: Sequences of the specimens used in the molecular identification.

PUTATIVE SPECIES	SEQUENCES
Clubiona sp.1	CAGAAAAAGAAGTATTAAAATTACGATCAGTCAATAATATAGTAATAGCTCCTGCTAAAACAGGTAAAGATAAAAG TAATAAAATAGCAGTAATCAAGACAGATCATACAAATAAAGGAACTTTCTTCCATAGTGATACCAACGGAGCGTAT ATTAACAATTGTGGTAATAAAATTCACAGCTCCTATAATAGAAGATGCACCAGCGAAATGAGGAGAAAAAATAGCA AAATCTATAGCACTTCCTATATGACCAACTGAAGACGCAAGCGGAGGATATACAGTTCAACCAGCTCCAACTCCTAT TTCAGCTATAGAAGAGATGAATAATAATAATAAAGAAGGAGGTAATAATCAAAAACTTAAGTTATTTATACGAGGA AAAGCCATATCAGCAGCTCCTAATATTATAGGAACTATTCAATTTCCAAAACCTCCAATTAAAATTGGTATTACTATA AAAAAGATTATAATAAATGCATGAGCCGTAACCACTACATTATATAAATGATCATCACCCAATAAAGCACCAGACTG TCCTAATTCTATACGAATTAATACTCTTATAGCAGTACCCACTATAGCAGATCGTGCTCCAAAAATTAAATACAAAGT TCCAATATCTTTATGATTTGTTGACCAACCAA
Cheiracanthium sp.	TGTATTATAATTTCGCATCACGTGTAATAATATAGTGAATGGCACCTGCTAATACAGGCAATGATAATAATAATAAC ACAGGCCGGAATCAATACAGAGTCACACAAATAAAGGTACTTTTTCTATAGGTATTCTTAGTAAACGTATATTAATA ATAGGAGAAATAAAATTAATAGCTCCTATAATTGAAGAAATTCCTGCTAAATGTAAAGAAAAAATAGCAAAATCAA CAGAACTCCCCGCATGACCTATAACAGAAGCCAAAGGAGGATACACTGTTCATCCAGCGCCCAACTCCTATTTCAAC TATAGAATAAATAAACAACAAGAATAGAGATGGGGGTAATAATCAAAAACTTAAATTATTTATTCGAGGAAAAGCT ATATCAGGAGCCCCTAATATTAAAGGAACCATTCAATTTCCAAAACCTCCAATTAATATTGGTATTACTATAAAAAAA ATCATAACAAAAGCATGAGCAGTTACTACAACATTGTACAACTGATCATCTCCTAAAAAAGACGCAACTTGCCCTAA TTCCATTCGAATTATAATTCTTATAGCCGTTCCAGCTATAGCTGATCAAGCCCCAAATATAAAATATAAAGTTCCAAT ATCTTTATGA
Meta sp.	AAAGGATGTATTAAAATTTCGATCTGTTAATAATATAGTAATAGCTCCTGCTAATACAGGTAAAGACAATAATAATA ATACAGCAGTAATTAAAACAGATCAAACAAATAAAGAAACTTTCTCTATAGTTATACCATAAGATCGTATATTTAAA ATAGTTCTAATAAAATTTACCGCCCCTATAATTGAAGAAGCTCCAGCTAAGTGAAGAGAAAAAATAGCAAAATCCAT TGATCTTCCTGAATGACCTTCAAGAGAAGATAAAGGAGGATATACTGTCCAACCTGCACCTACACCCATTTCTACTA TAGAAGAAATAAATAATAAAATTAAAGAAGGTGGTAATAATCAAAATCTAAGATTATTTATACGAGGAAAAGCCAT ATCAGGAGCACCTAATATTATAGGAACTAATCAATTTCCAAATCCACCAATTAGAATTGGTATAACTATAAAAAAAA TTATAACAAAAGCATGACTAGTGACAATAACATTATATAATTGATCATTACCTAAAAATCTACCTGGTTGTCCTAATT CAATTCGAATTAAAACTCTTATTGCAGTTCCTACTATAGCTGCTCATCTTCCAAAAATTAAATATAAAGTTCCAATATC TTTATGA
Tetragnatha sp.1	GTATTAAAATTTCGATCAGTTAATAATATAGTAATAGCACCAGCCAATACTGGTAAAGATAATAATAATAAAACAGC AGTAATCAATACAGATCAAACAAATAAAGGTACCTTTTCTATAGTCATTCCATAAAATCGTATATTAATAATAGTAGA AATAAAATTAATTGCACCCATAATCGATGAGGCCCCGGCTAAATGTAAAGAAAAAATTGCAAAATCTACTGATCTAC CAGCATGACCCTCTAATCCAGCTAAAGGTGGATAAACCGTTCAACCAGCACCAACACCTATTTCAACTATAGAAGAA ATAATTAATAAAAATAAAGAAGGAGGTAATAATCAAAATCTTAAATTATTTATTCGAGGAAATGCTATGTCGGGAG CCCCTAACATTAATGGAACTAATCAATTTCCAAATCCCCCAATTAAAATTGGTATTACTATAAAAAAAATTATTACAA ATGCATGAGCCGTTACAATTACATTATATAATTGATCATCACCTAAAAATCTTCCTGGCTGACCTAACTCAATTCGAA TCAATACTCTTATTGCTGTCCCTACTATAGCAGCCCAAGCCCCAAAAATCAAATATAAAGTTCCAATATCTTTATGATT TTGTTGACCAAA
Camponotus lateralis	CGAAGAAAGAGGTATTAAGATTTCGATCTGTTAATAATATAGTAATAGCCCCAGCTAATACAGGTAGGGATAGAAG GAGAAGGATGGCTGTGATAAGAATTGATCATACTAATAAAGGAATTTTATCTATAGAGATATTGATATTATGTATAT TTAAAATTGTTGAGATAAAATTGATTGCCCCTAAGATAGAGGATATACCTGCAATGTGGAGGGAGAAGATAGTTAA

PUTATIVE SPECIES	SEQUENCES
	ATCTACAGAGGGGCCACTATGAAAAGTATTAGATGATAGAGGGGGATAAATTGTTCAACCTGTTCCAGATCCTTCA TTAATAAAATTTCTTAGTATTAGGAGAGAGATTGAGGGGGGTAATAATCAAAATCTTATATTATTTAGACGAGGGT AGGCCATATCAGGAGAACCTAATATTAATGGGATTAAAAAATTACCAAATCCTCCGATTATGAAGGGTATAACTATG AAAAAGATTATAATAAAAGCATGACCTGTTACAATGGAGTTAAAGGTTTGATCATTAGAAATTAATGAATTGGGAG ATCCTAATTCTAATCGAATAATTATTCTTATTGATGAACCAATTATCCCAGATCAAATTGCAAAGATAAAGTATAAGA TTCCAATATCTTTATGATTTTGGTTGACC
Crematogaster auberti	AATAGTCGGCTCATCTATAAGTATAATCATTCGATTAGAATTGGGATCATGCGATTCTTTAATTGGTAATGATCAAAT TTTTAATGTCCTAGTTACCGGACATGCTTTTATTATAATTTTCTTTATAGTTATGCCATTTATAATCGGCGGATTCGGA AATTTCCTCGTTCCTCTTATACTTGGCTCTCCCGACATAGCTTATCCACGTATAAATAATATAAGATTCTGACTCTTAC CCCCATCAATTTTATTACTTCTTCTTAGAGGATTTATTAATACAGGAGTAGGGACCGGATGAACTATCTATCCTCCTTT AGCCTCAAATATTTTTCACAGAGGACCGTCAACTGATCTCTCAATCTTTTCCCTTCATATTGCTGGTATATCATCAATT TTAGGAGCTATTAATTTTATTGCAACTATCTTAAATATACATCATAAATCCTTTTCCCTAGATAAAATTTCCCTTTTAAC CTGATCTATCCTTATTACTGCTATCCTTCTCCTTCTTTCTTTACCAGTTTTAGCTGGAGCAATTACTATATTATTAACAG ACCGTAATCTTAATACTTCCTTCTTTGACCCCTCAGGAGGAGGCGACCCTATTTTATATCAACATCTTTTTTGATTTTT TGGTCACCTGGAAGTTTAA
Crematogaster scutellaris	CGAAGAATGAAGTATCTAAGATTACGGTCTGTTAATACTATATTAATTGCTCCAGCTAAAACTGGTAAAGAAAGAA GGAGAAGGATAGCAGTAATAAGGATAGATCAGGTTAAAAGGGAAATTTTATCTAGGGAAAAGGATTTATGATGTA TATTTAAGATAGTTGCAATAAAATTAATAGCTCCTAAAATTGATGATATACCAGCAATATGAAGGGAAAAGATTGA GAGATCAGTTGACGGTCCTCTGTGAAAAATATTTGAGGCTAAAGGAGGATAGATAGTTCATCCGGTCCCTACTCCT GTATTAATAAATCCTCTAAGAAGAAGTAATAAAATTGATGGGGGTAAGAGTCAGAATCTTATATTATTTATACGTGG ATAAGCTATGTCGGGAGAGCCAAGTATAAGAGGAACGAGGAAATTTCCGAATCCGCCGATTATAAATGGCATAACT ATAAAGAAAATTATAATAAAAGCATGTCCGGTAACTAGGACATTAAAAATTTGATCATTACCAATTAAAGAATCGCA TGATCCCAATTCTAATCGAATGATTATACTTATAGATGAGCCGACTATCCCAGCTCAAATAGCAAAGATAAAATATA AAATTCCAATATCTTTATGATTTGTTGACC
Crematogaster sordidula	AATAATTGGCTCATCTATAAGAATAATTATTCGATTAGAATTAGGATCATGTGACTCACTAATTAATAATGATCAAAT TTACAACTCTATAGTTACAAGCCACGCTTTTATTATAATTTTTTTTATAGTAATACCTTTCATAATTGGGGGATTTGGA AATTTTCTTGTCCCCCTCATACTAGGCTCACCGGATATAGCCTATCCACGTATAAATAATATAAGATTTTGACTCTTGC CCCCCTCTATTACTCTCCTTTTATTAGGAAGATTTATTAACTCAGGGGCCGGTACTGGGTGAACAGTTTACCCCCCAT TAGCCTCCAATATTTTCCACAGAGGAGCTTCTATCGATCTATCCATCTTTTCCCTTCACATCGCAGGTATATCCTCCAT CCTAGGAGCTATCAATTTTATTTCTACGATTATTAATATACATCATAAAAGTTTCTCCATAGACAAAATTCCATTATTA GTCTGATCAATCCTTATCACTGCAATTTTACTTCTTCTTTCCCTCCCAGTCCTTGCAGGAGCCATTACTATACTTCTCAC GGACCGAAATTTAAATACTTCCTTCTTCGACCCTGCAGGAGGAGGAGACCCCATCCTATACCAACACCTATTCTGAT TTTTTGGTCACCTGGAAAGTTTAA
Iberoformica subrufa	CAAAAAATGAGGTATTTAAATTTCGATCAGTTAATAATATAGTAATAGCTCCAGCTAACACAGGTAAAGATAATAAT AATAAAATTGCAGTAATTAAAATTGATCATACTAAAAGGGGAATTTTATCAATAGAAAAATTTTTATGATGTATATTA ATAATTGTTGAAATAAAATTAATTGCACCTAAGATCGATGATATACCAGCAATATGAAGAGAAAAAATTGTTAAATC TACCGAAGGTCCATTATGAAAAATATTAGAGGCTAAAGGAGGGGTATACTGTTCATCCTGTTCCTGTACCGTCATTAA TAAAGTTACTTAATAATAAAAGAGTAATAGAGGGCGGTAATAGTCAAAATCTTATATTGTTTATTCGAGGATAAGCT ATATCTGGGGAACCTAATATTAAGGGGATTAAAAAATTTCCAAATCCACCAATTATAAATGGTATAACTATAAAGAA AATTATAATAAATGCGTGATTAGTAACTATAGAATTATAGATTTGATCATTTCTAATTAATGAGTTTGAGGACCCTAG TTCCAAACGAATAATTATACTTATAGAAGATCCAATTATTCCGGATCAAATAGCGAATATAAAATAAAGAATACCAA TATCTTTATGATTTTGTTGAC
Lasius brunneus	CAAAGAATGATGTATTTAGATTTCGGTCAGTTAAAAGTATTGTAATTGCTCCTGCTAAAACAGGTAAAGATAAAAGC AATAATACAGCTGTAATTAGAATTGATCAAACTAAAAGAGGGATTTTGTCAATAGAAAAATTTTTATGGTGTATATT TAGAATTGTTGAGATAAAATTGATTGCCCCTAAAATAGAAGATATTCCTGCAATGTGTAAAGAGAAGATGGCTAAA TCAACGGATGGTCCGCTATGGTATATGTTTGCTGCTAAAGGAGGATAAACTGTTCACCCTGTACCAACACCTGTGCC AATAAAATTTCTAATAGTTAAAAGTAAAATTGAAGGGGGTAATAATCAAAATCTTATATTATTTATTCGAGGGTATG CTATATCTGGGGCTCCTAATATTAGTGGTACTAAGAAATTACCAAAACCACCAATTATAAAGGGCATTACTATAAAG AAAATTATAATAAATGCATGTCCTGTTACAATTGAGTTATAAATTTGATCATTTATAATTAGAGAATTACATGTTCCT AATTCTAATCGAATGATTATTCTTATTGATGACCCAATTATTCCGGATCAAATGGCAAAAATAAAATATAATATTCCA ATATCTTTATGATTTTGTTGACC
Plagiolepis schmitzi	TCAGTAAGAAGTATAGTGATAGCCCCAGCTAGAACAGGAAGAGAGAGAAGGAGAAGAATAGCTGTAATGAACAT AGATCAGACTAATAGAGGAATTTTGTCTATAGAAGAATTTTTATGATGTATATTTAAGATAGGTGAAATAAAATTGA TAGCCCCTAAAATAGAGGACATTCCAGCAATATGAAGGGAAAAAATAGTTAGATCAACTGAAGGGCCATTATGAAA AATATTAGAAGCTAGAGGAGGATAAATAGGTCATCCTGTCCCTACTCCATCATTAATAAAATTTCTTAAAAGTAGTA AAGAAATAGAAGGGGGTAAAAGTCAAAATCTTATATTATTTATTCGTGGATAGGCTATGTCGGGAGATCCAATTAT AAGAGGAATTAAGAAATTTCCAAATCCGCCAATTATAAAAGGTATAACCATAAAAAAAATTATAATACATGCGTGA CTTGTGACTATAGAATTATAAATTTGATCATTACTAATTAATGCGTTAGAAGATCCTAGTTCTAGACGAATAATTATT CTTATAGATGAGCCTAATATTCCTGCGCACATAGCACATAAGAGATATAAAATTCCAATATCTTTATGAGATGTTGA CCA

PUTATIVE SPECIES	SEQUENCES
Tapinoma sp.1 (nigerrimum-simrothi complex)	ATTTCGGTCAGTTAATAATATAGTAATTGCCCCAGCTAAAACTGGAAGTGATAAAAGTAATAAAATTGCAGTAATTA AAATTGATCAAACTAATAAAGGAATTTTATCAATAGAAAAATTTTTATGATGTATATTAATAATTGTGGAAATAAAAT TAATAGCTCCTAAAATTGATGATATACCTGCAATATGTAAAGAAAAAATAGCTAAATCTACTGAGGGCCCATTGTGA AAAATATTAGATGCTAAGGGGGGATATACAGTCCAACCGGTCCCAACCCCTGAACCAATAAAATTACTGATAGTTA ATAATAAAATTGAGGGGGGTAATAATCAAAATCTTATATTATTTATTCGTGGGTAAGCTATATCTGGAGCCCCTAAT ATTAAAGGTACTAAAAAATTACCAAATCCACCAATTATAAAAGGTATAACTATAAAAAAAATTATAACAAAAGCATG ACCTGTAACAATTGAATTATAAATTTGATCATTATTAATTAATGCCCCGCATGTTCCTAATTCAATACGAATAATTATC CTTATTGATGAGCCAACTATTCCTGATCAAATAGCAAAAATAAAATATAATAAACCAATATCTTTATGA

Table A5: Samples codes and their localization within the Alentejo region.

QUADRANT	POINT	HOST	CODE	LATITUDE	LONGITUDE
0	153	Oli	Q0P153OLI	38.98953	-8.29402
0	155	Oli	Q0P155OLI	38.99588	-8.36109
0	153N	Oli	Q0P153NOLI	39.01608	-8.27235
0	153N	Esp	Q0153NESP	39.01608	-8.27235
1	145	Oli	Q1P145OLI	38.86915	-8.15131
1	146	Oli	Q1P146OLI	38.87482	-8.15239
1	146	Esp	Q1P146ESP	38.87482	-8.15239
1	147	Oli	Q1P147OLI	38.87367	-8.15403
1	148	Oli	Q1P148OLI	38.94074	-8.13174
1	148	Esp	Q1P148ESP	38.94074	-8.13174
1	149	Esp	Q1P149ESP	38.94808	-8.06918
1	150	Oli	Q1P150OLI	38.9672	-8.10406
1	151	Esp	Q1P151ESP	39.04548	-8.21453
2	160	Oli	Q2P160OLI	38.92902	-7.8099
2	161	Oli	Q2P161OLI	39.00259	-7.79916
2	162	Oli	Q2P162OLI	39.03954	-7.80783
2	162	Esp	Q2P162ESP	39.03954	-7.80783
2	163	Oli	Q2P163OLI	39.04812	-7.65475
2	163	Esp	Q2P163ESP	39.04812	-7.65475
2	164	Oli	Q2P164OLI	39.03892	-7.6569
2	164	Esp	Q2P164ESP	39.03892	-7.6569
2	165	Oli	Q2P165OLI	38.97609	-7.67723
2	166	Oli	Q2P166OLI	38.91774	-7.66367
2	166	Esp	Q2P166ESP	38.91774	-7.66367
2	168	Oli	Q2 P168 OLI	38.94825	-7.49201
2	168	Esp	Q2P168ESP	38.94825	-7.49201
3	169	Oli	Q3P169OLI	38.92109	-7.42266
3	170	Oli	Q3P170OLI	38.91549	-7.32053
3	170	Esp	Q3P170ESP	38.91549	-7.32053
3	171	Oli	Q3P171OLI	38.96419	-7.29419
3	185	Oli	Q3P185OLI	39.01338	-7.25501
4	157	Oli	Q4P157OLI	38.9079	-7.1453
4	157	Esp	Q4P157ESP	38.9079	-7.1453
4	174	Oli	Q4P174OLI	38.90101	-7.1338

4	174	Esp	Q4P174ESP	38.90101	-7.1338
4	175	Oli	Q4P175OLI	38.88699	-7.15092
4	180	Oli	Q4P180OLI	39.01118	-7.08055
4	180	Esp	Q4P180ESP	39.01118	-7.08055
4	181	Oli	Q4P181OLI	39.03364	-7.08799
4	181	Esp	Q4P181ESP	39.03364	-7.08799
4	183	Oli	Q4P183OLI	39.0094	-7.1093
4	183	Esp	Q4P183ESP	39.0094	-7.1093
4	183B	Oli	Q4P183BOLI	39.0094	-7.1093
6	112	Oli	Q6P112OLI	38.6059	-8.45219
6	113	Oli	Q6P113OLI	38.68583	-8.50106
6	113	Esp	Q6P113ESP	38.68583	-8.50106
6	114	Oli	Q6P114OLI	38.68508	-8.48567
6	114	Esp	Q6P114ESP	38.68508	-8.48567
6	115	Oli	Q6P115OLI	38.67975	-8.4649
6	115	Esp	Q6P115ESP	38.67975	-8.4649
6	116	Oli	Q6P116OLI	38.71969	-8.43084
6	117	Oli	Q6P117OLI	38.77359	-8.36852
6	117	Esp	Q6P117ESP	38.77359	-8.36852
6	118	Oli	Q6P118OLI	38.73648	-8.33528
6	118	Esp	Q6P118ESP	38.73648	-8.33528
7	119	Oli	Q7P119OLI	38.71844	-7.98973
7	119	Esp	Q7P119ESP	38.71844	-7.98973
7	120	Oli	Q7P120OLI	38.79414	-7.96899
7	121	Oli	Q7P121OLI	38.79577	-7.93814
7	122	Oli	Q7P122OLI	38.8	-7.91698
7	122	Esp	Q7P122ESP	38.8	-7.91698
7	123	Oli	Q7P123OLI	38.79621	-7.89404
7	123	Esp	Q7P123ESP	38.79621	-7.89404
7	143	Oli	Q7P143OLI	38.65526	-8.20524
7	143	Esp	Q7P143ESP	38.65526	-8.20524
7	144	Oli	Q7P144OLI	38.69515	-8.20032
7	144	Esp	Q7P144ESP	38.69515	-8.20032
8	124	Oli	Q8P124OLI	38.7056	-7.78687
8	126	Oli	Q8P126OLI	38.6972	-7.77901
8	128	Oli	Q8P128OLI	38.76606	-7.7135
8	129	Oli	Q8P129OLI	38.77226	-7.71758
8	129	Esp	Q8P129ESP	38.77226	-7.71758
8	131	Esp	Q8P131ESP	38.8338	-7.64151
8	159	Oli	Q8P159OLI	38.81987	-7.82594
8	127N	Oli	Q8P127NOLI	38.75524	-7.72921
9	132	Oli	Q9P132OLI	38.76937	-7.4153
9	133	Oli	Q9P133OLI	38.73793	-7.40356
9	134	Oli	Q9P134OLI	38.7016	-7.40901
9	134	Esp	Q9P134ESP	38.7016	-7.40901
9	137	Oli	Q9P137OLI	38.61179	-7.34816

9	139	Oli	Q9P139OLI	38.6675	-7.32894
9	141	Oli	Q9P141OLI	38.73635	-7.26912
9	142	Oli	Q9P142OLI	38.74632	-7.23827
9	142	Esp	Q9P142ESP	38.74632	-7.23827
13	100	Oli	Q13P100OLI	38.52527	-8.24082
13	101	Oli	Q13P101OLI	38.53549	-8.15461
13	102	Oli	Q13P102OLI	38.52644	-8.13569
13	102	Esp	Q13P102ESP	38.52644	-8.13569
13	103	Oli	Q13P103OLI	38.52666	-8.01599
13	104	Oli	Q13P104OLI	38.38506	-8.1685
13	105	Oli	Q13P105OLI	38.38302	-8.17132
13	102C	Oli	Q13P102COLI	38.5261	-8.10235
14	79	Oli	Q14P79OLI	38.46269	-7.76131
14	95	Oli	Q14P95OLI	38.5078	-7.56616
14	97	Oli	Q14P97OLI	38.50308	-7.60025
14	98	Oli	Q14P98OLI	38.45461	-7.63268
14	98	Esp	Q14P98ESP	38.45461	-7.63268
14	99	Oli	Q14P99OLI	38.4944	-7.74908
14	79B	Oli	Q14P79BOLI	38.45565	-7.76247
14	79B	Esp	Q14P79BESP	38.45565	-7.76247
14	96N	Oli	Q14P96NOLI	38.50313	-7.60157
15	84	Oli	Q15P84OLI	38.38202	-7.33151
15	86	Oli	Q15P86OLI	38.44892	-7.39888
15	88	Oli	Q15P88OLI	38.4465	-7.49286
15	89	Oli	Q15P89OLI	38.47992	-7.51632
15	91	Oli	Q15P91OLI	38.52991	-7.49247
15	92	Oli	Q15P92OLI	38.54418	-7.48383
15	136	Oli	Q15P136OLI	38.57861	-7.3568
19	65	Oli	Q19P65OLI	38.24448	-7.94566
19	65	Esp	Q19P65ESP	38.24448	-7.94566
19	67	Oli	Q19P67OLI	38.23446	-8.00726
19	67	Esp	Q19P67ESP	38.23446	-8.00726
19	69	Oli	Q19P69OLI	38.08581	-8.06244
19	70	Oli	Q19P70OLI	38.09587	-8.13166
19	71	Oli	Q19P71OLI	38.16677	-8.1435
19	73	Oli	Q19P73OLI	38.23583	-8.20925
19	73	Esp	Q19P73ESP	38.23583	-8.20925
19	106	Oli	Q19P106OLI	38.30796	-8.22722
20	57	Oli	Q20P57OLI	38.29573	-7.70544
20	58	Oli	Q20P58OLI	38.11885	-7.63822
20	59	Oli	Q20P59OLI	38.14203	-7.75794
20	60	Oli	Q20P60OLI	38.12144	-7.83733
20	61	Oli	Q20P61OLI	38.19842	-7.85679
20	62	Oli	Q20P62OLI	38.21461	-7.83039
20	81	Oli	Q20P81OLI	38.31715	-7.69828
21	38	Oli	Q21P38OLI	38.09468	-7.2542

21	39	Oli	Q21P39OLI	38.22335	-7.33959
21	39	Esp	Q21P39ESP	38.22335	-7.33959
21	48	Oli	Q21P48OLI	38.13128	-7.32953
21	49	Oli	Q21P49OLI	38.13595	-7.4364
21	52	Oli	Q21P52OLI	38.15657	-7.42575
21	55	Oli	Q21P55OLI	38.21823	-7.54243
25	1	Oli	Q25P1OLI	38.03523	-8.11084
25	2	Oli	Q25P2OLI	37.97444	-8.0858
25	3	Oli	Q25P3OLI	37.97352	-8.08255
25	3	Esp	Q25P3ESP	37.97352	-8.08255
25	4	Oli	Q25P4OLI	37.94581	-8.09712
25	6	Oli	Q25P6OLI	37.8822	-8.1581
25	6	Esp	Q25P6ESP	37.8822	-8.1581
25	7	Esp	Q25P7ESP	37.87733	-8.15344
25	8	Oli	Q25P8OLI	37.82484	-8.10576
25	8	Esp	Q25P8ESP	37.82484	-8.10576
26	11	Oli	Q26P11OLI	37.96064	-7.8068
26	13	Oli	Q26P13OLI	38.02918	-7.77964
26	15	Oli	Q26P15OLI	38.01324	-7.70896
26	16	Oli	Q26P16OLI	37.96109	-7.617
26	18	Oli	Q26P18OLI	37.9513	-7.58702
27	19	Oli	Q27P19OLI	37.92152	-7.45526
27	20	Oli	Q27P20OLI	37.91983	-7.43679
27	21	Oli	Q27P21OLI	37.90078	-7.40088
27	23	Oli	Q27P23OLI	37.94351	-7.31875
27	24	Oli	Q27P24OLI	37.94482	-7.31671
27	24	Esp	Q27P24ESP	37.94482	-7.31671
27	25	Oli	Q27P25OLI	38.02612	-7.3005
27	25	Esp	Q27P25ESP	38.02612	-7.3005
27	22N	Oli	Q27P22NOLI	37.9202	-7.4076